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(54) Title: THERMOSTABLE PHYTASES IN FEED PREPARATION AND PLANT EXPRESSION

(57) Abstract

The use of thermostable phytases in the preparation of animal feed, and the expression in plants of such phytases. For preparation of animal feed, a thermostable phytase is added before or during the agglomeration step. Preferred processes are pelleting, extrusion and expansion. A transgenic plant expressing a thermostable phytase may be used diretly in animal feed preparation.

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# Thermostable phytases in feed preparation and plant expression

## Technical Field

This application relates to thermostable phytases, viz. their use in processes for the production of animal feed, and their expression in plants.

## Background art

WO 91/14782 describes transgenic tobacco and rapeseed plants expressing a phytase derived from Aspergillus ficuum NRRL 3135. The transgenic tobacco seeds are fed to broilers.

US 5,824,779 describes in standard fashion how to produce transgenic alfalfa expressing the same A. ficuum phytase, and the preparation of a phytase-containing concentrate which can be used per se as an animal feed supplement.

EP 0 556 883 B1 describes a method for preparing feed pellets based on an extrusion technique. The addition of temperature sensitive agents, one example of which is phytase, takes place after extrusion of the feed pellets, and the sensitive agents are loaded onto the pellets under reduced pressure.

As acknowledged in EP 0 556 883 B1 the loss of activity of heat-sensitive substances during feed preparation processes is a well-known problem. The above EP-patent proposes to solve this problem by adding these substances under reduced pressure subsequent to the extrusion process. This solution, however, requires a liquid form of the sensitive substance, as well as the installation of additional expensive process equipment.

The present invention provides an improved process for preparing animal feed, as well as improved phytase-expressing transgenic plants.

**Summary of the Invention**

The present invention provides a process of preparing an animal feed, which process comprises an agglomeration of feed ingredients, wherein a thermostable phytase is added before or during the agglomeration.

Also provided is a transgenic plant or part thereof which comprises a DNA-construct encoding a thermostable phytase.

The transgenic plant or part thereof, e.g. seeds or leaves, may be used in the feed preparation process of the invention, to thereby provide - in a preferred embodiment - at the same time a nutrient (feed ingredient) and the feed additive phytase.

**Brief description of the Figures**

In the detailed description of the invention below, reference is made to the drawings, of which

| | |
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| Fig. 1 | is a differential scanning calorimetry (DSC) chart of consensus phytase-1 and consensus phytase-10; |
| Fig. 2 | a DSC of consensus phytase-10-thermo-Q50T and consensus phytase-10-thermo-Q50T-K91A; |
| Fig. 3 | a DSC of consensus phytase-1-thermo[8]-Q50T and consensus phytase-1-thermo[8]-Q50T-K91A; |
| Fig. 4 | a DSC of the phytase from A. fumigatus ATCC 13073 and of its α-mutant; and |
| Fig. 5 | shows the design of the consensus-phytase-1 amino acid sequence; |

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| Fig. 6 | an alignment and the basidiomycete consensus sequence of five Basidiomycete phytases; |
| Fig. 7 | the design of the consensus-phytase-10 amino acid sequence; |
| Fig. 8 | an alignment for the design of consensus-phytase-11 (all Basidiomycete phytases were used as independent sequences using an assigned vote weight of 0.2 for each Basidiomycete sequence; still further the amino acid sequence of A. niger T213 was used); |
| Fig. 9 | the DNA and amino acid sequence of consensus-phytase-1-thermo(8)-Q50T-K91A; |
| Fig. 10 | the DNA and amino acid sequence of Consensus-phytase-10-thermo(3)-Q50T-K91A; |
| Fig. 11 | the DNA and amino acid sequence of A. fumigatus ATCC 13073 α-mutant; and |
| Fig. 12 | the DNA and amino acid sequence of Consensus-phytase-7 which comprises the following mutations as compared to Consensus-phytase-1: S89D, S92G, A94K, D164S, P201S, G203A, G205S, H212P, G224A, D226T, E255T, D256E, V258T, P265S, Q292H, G300K, Y305H, A314T, S364G, M365I, A397S, S398A, G404A, and A405S. |

## Detailed description of the invention

In the present context a "feed" or an "animal feed" means any natural or artificial diet, meal or the like intended or suitable for being eaten, taken in, digested, by an animal. Food for human beings is included in the above definition of feed.

"Animals" include all animals, be it polygastric animals (ruminants); or monogastric animals such as human beings,

poultry, swine and fish. Preferred animals are the mono-gastric animals, in particular pigs and broilers.

The concept of "feed ingredients" includes the raw materials from which a feed is to be, or is, produced; or the intended, or actual, component parts of a feed. Feed ingredients for non-human animals are usually, and preferably, selected from amongst the following non-exclusive list:

plant derived products

such as seeds, grains, leaves, roots, tubers, flowers, pods, husks - and they may take the form of flakes, cakes, grits, flour, and the like;

animal derived products

such as fish meal, milk powder, bone extract, meat extract, blood extract and the like;

additives

such as minerals, vitamins, aroma compounds, and feed enhancing enzymes.

Phytic acid or myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate (or for short myo-inositol hexakisphosphate) is the primary source of inositol and the primary storage form of phosphate in plant seeds and grains. In the seeds of legumes it accounts for about 70% of the phosphate content. Seeds, cereal grains and legumes are important feed ingredients.

Phytic acid, or its salts phytates - said terms being, unless otherwise indicated, in the present context used synonymously or at random - is an anti-nutritional factor. This is partly due to its binding of nutritionally essential ions such as calcium, trace minerals such as mangane, and also proteins (by electrostatic interaction). And partly due to the fact that the phosphorous thereof is not nutritionally available

either, since phytic acid and its salts, phytates, are often not metabolized.

This leads to a need of supplementing food and feed preparations with e.g. inorganic phosphate.

The non-metabolizable phytic acid phosphorous passes through the gastrointestinal tract of such animals and is excreted with the manure, resulting in an undesirable phosphate pollution of the environment resulting e.g. in eutrophication of the water environment and extensive growth of algae.

Phytic acid is degradable by phytases. In the present context a "phytase" is an polypeptide or enzyme which exhibits phytase activity, viz. which catalyzes the hydrolysis of phytate (myo-inositol hexakisphosphate) to (1) myo-inositol and/or (2) mono-, di-, tri-, tetra- and/or penta-phosphates thereof and (3) inorganic phosphate.

The production of phytases by plants as well as by microorganisms has been reported. Amongst the microorganisms, phytase producing bacteria as well as phytase producing fungi are known.

There are several descriptions of phytase producing filamentous fungi belonging to the fungal phylum of Ascomycota (ascomycetes). In particular, there are several references to phytase producing ascomycetes of the Aspergillus genus such as Aspergillus terreus (Yamada et al., 1986, Agric. Biol. Chem. 322:1275-1282). Also, the cloning and expression of the phytase gene from Aspergillus niger var. awamori has been described (Piddington et al., 1993, Gene 133:55-62). EP 0420358 describes the cloning and expression of a phytase of Aspergillus ficuum (niger). EP 0684313 describes the cloning and expression of phytases of the ascomycetes Aspergillus niger, Myceliophthora thermophila, Aspergillus terreus. Still further, some partial

sequences of phytases of Aspergillus nidulans, Talaromyces thermophilus, Aspergillus fumigatus and another strain of Aspergillus terreus are given.

The cloning and expression of a phytase of Thermomyces lanuginosus is described in WO 97/35017.

WO 98/28409 describes the cloning and expression of several basidiomycete phytases, e.g. from Peniophora lycii, Agrocybe pediades, Paxillus involutus and Trametes pubescens.

According to the Enzyme nomenclature database ExPASy (a repository of information relative to the nomenclature of enzymes primarily based on the recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB) describing each type of characterized enzyme for which an EC (Enzyme Commission) number has been provided), two different types of phytases are presently known: A so-called 3-phytase (myo-inositol hexaphosphate 3-phosphohydrolase, EC 3.1.3.8) and a so-called 6-phytase (myo-inositol hexaphosphate 6-phosphohydrolase, EC 3.1.3.26). The 3-phytase hydrolyses first the ester bond at a 3-position, whereas the 6-phytase hydrolyzes first an ester bond at the 6-position of phytic acid. Both of these types of phytases are included in the above definition of phytase.

Many assays of phytase activity are known, and any of these can be used for the purpose of the present invention. Preferred phytase assays are included in the examples.

The concept of "agglomeration" is defined as a process in which various components are mixed under the influence of heat. The resulting product is preferably an "agglomerate" or conglomerate in which the components adhere to each other while forming a product of a satisfactory physical stability. The formation of dust from such agglomerate is an indication of its

physical stability - the less dust being formed, the better. A suitable assay for dust formation from agglomerates is ASAE standard S 269-1. A satisfactory agglomerate has below 20%, preferably below 15%, more preferably below 10%, even more preferably below 6% dust.

"Under the influence of heat" means that the temperature is at least 65°C, as measured on the product at the outlet of the agglomeration unit. More preferred temperatures are at least 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, or even at least 130°C.

A preferred agglomeration process is operated at an increased pressure. The pressure is typically due to a compacting of the ingredients, optionally in combination with a reduction of the cross-sectional or throughput area. Preferably, by properly adjusting process parameters such as temperature and pressure, the resulting shear forces and shear velocities are of such magnitude, that the starch- and protein-containing feed ingredients become fluid.

"Increased pressure" means increased as compared to normal atmospheric pressure, and the maximum pressure as measured within the agglomeration unit.

The addition of water vapour or steam is often included in agglomeration, but not as an absolute requirement.

Agglomeration includes, but is not limited to, the well-known processes called extrusion, expansion (or pressure conditioning) and pelleting (or pellet pressing).

Extrusion is i.a. described at pp. 149-153 of a handbook which is available on request from the Danish Company Sprout-Matador, Glentevej 5-7, DK-6705 Esbjerg Ø or Niels Finsensvej 4, DK-7100 Vejle ("Håndbog i Pilleteringsteknik 1996"). However, in the agglomeration process of the invention, the following

process steps mentioned in the above handbook are entirely optional:

(i) pre-treating the feed ingredients in a cascade mixer;
(ii) cutting the product leaving the nozzle-section into pieces
(iii) of a desired size;
(iv) acclimatizing or conditioning it;
(v) coating it;
(vi) drying it;
(vii) cooling it.

The process of expansion (pressure conditioning) is i.a. described in the same handbook at pp. 61-66. Also for expansion, the above process steps (i)-(vi), in particular steps (i) and (vi), are entirely optional steps.

This is so also for the following process steps:

(ii') comminuting the product (using e.g. a blade granulator as shown at p. 65);

(vii) pelleting the product (using e.g. a pellet press as shown at p. 62);

The process of pelleting is i.a. described in the same handbook at pp. 71-107. Also here, steps (i)-(vii) above are entirely optional steps. These steps are i.a. described in more detail at pp. 29-70 of the above handbook.

In a preferred agglomeration process of the invention, one or more of the above mentioned further process steps (i)-(vii) are included.

A particularly preferred further step is step (i).

In a most preferred embodiment, the feed-ingredients are pre-heated in a first step (a) to a temperature of at least 45°C, preferably at least 50, 55, 60, 65, 70, 75, 80 °C; and then heated in a second step (b) to a temperature of at least

65°C, preferably 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, or even at least 130°C.

The addition of thermostable phytase takes place before or during step (a) and/or before or during step (b).

Water is preferably added in step (a). More preferably, heated steam is added during the mixing of the ingredients (steps (a) and/or (b)).

Process step (a) is preferably performed in a cascade mixer (see the above cited handbook p. 44).

A "thermostable" phytase is a phytase which has a Tm (melting temperature) as measured on purified phytase protein by Differential Scanning Calorimetry (DSC) of at least 65°C, preferably using for the DSC a constant heating rate, more preferably of 10°C/min. In preferred embodiments, the Tm is at least 66, 67, 68, 69, 70, 71, 72, 73, 74 or 75°C. Preferably, the Tm is equal to or lower than 150°C, more preferably equal to or lower than 145, 140, 135, 130, 125, 120, 115 or 110°C. Accordingly, preferred intervals of Tm are: 65-150°C, 66-150°C, - (etc.) - 75-150°C; 65-145°C, 66-145°C, - (etc.) - 75-145°C; 65-140°C, - (etc.) - 75-140°C; - (etc.) - 65-110°C, 66-110°C, - (etc.) - 75-110°C.

Particularly preferred ranges for Tm are the following: between 65 and 110°C; between 70 and 110°C; between 70 and 100°C; between 75 and 95°C, or between 80 and 90°C.

In Example 3 below, the measurement of Tm by DSC is described, and the Tm's of a number of phytases are shown.

The optimum temperatures are also indicated, since - in the alternative - a thermostable phytase can be defined as a phytase having a temperature-optimum of at least 60°C. Preferably, the optimum temperature is determined on the substrate phytate at pH 5.5, or on the substrate phytic acid at

pH 5.0. Preferred units are FYT, FTU or the units of Example 3. The phytase assay of Example 3 is most preferred.

In preferred embodiments, the optimum temperature is at least 61, 62, 63, 64, 65, 66, 67, 68, 69 or 70°C. Preferably, the optimum temperature is equal to or lower than 140°C, more preferably equal to or lower than 135, 130, 125, 120, 115, 110, 105 or 100°C. Accordingly, preferred intervals of optimum temperature are: 60-140°C, 61-140°C, - (etc.) - 70-140°C; 60-135°C, 61-135°C, - (etc.) - 70-135°C; 60-130°C, - (etc.) - 70-130°C; - (etc.) - 60-100°C, 61-100°C, - (etc.) - 70-100°C.

Preferred phytases of the present invention exhibit a degree of similarity or homology, preferably identity, to the complete amino acid sequence of either of the phytases mentioned below under (iii) - preferably to the complete amino acid sequence of Consensus-phytase-10-thermo-Q50T-K91A - of at least 48%, preferably at least 50, 52, 55, 60, 62, 65, 67, 70, 73, 75, 77, 80, 82, 85, 88, 90, 92, 95, 98 or 99%.

The degree of similarity or homology, alternatively identity, can be determined using any alignment programme known in the art. A preferred alignment programme is GAP provided in the GCG version 8 program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711) (see also Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-453). Using GAP with the following settings for polypeptide sequence comparison: GAP weight of 3.000 and GAP lengthweight of 0.100.

A multiple sequence alignment can be made using the program PileUp (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science

Drive, Madison, Wisconsin, USA 53711), with a GapWeight of 3.000 and a GapLengthWeight of 0.100.

Using the program GAP, some selected phytases exhibit the following percentage similarity (identity in brackets) to the Consensus-phytase-10-thermo(3)-Q50T-K91A amino acid sequence:

| | |
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| A. fumigatus ATCC-13073 α-mutant | 86.7% (81.8%) |
| Basidiomycet consensus | 64.1% (49.0%) |
| Consensus-phytase-1 | 98.7% (97.9%) |
| Consensus-phytase-10 | 96.6% (94.4%) |
| Consensus-phytase-1-thermo(8)-Q50T-K91A | 97.4% (95.5%) |
| Consensus-phytase-11 | 96.5% (94.2%) |
| Consensus-phytase-12 | 92.5% (89.9%) |
| Consensus-phytase-7 | 95.5% (93.4%) |

A "purified" phytase is essentially free of other non-phytase polypeptides, e.g. at least about 20% pure, preferably at least about 40% pure, more preferably about 60% pure, even more preferably about 80% pure, most preferably about 90% pure, and even most preferably about 95% pure, as determined by SDS-PAGE.

Preferred thermostable phytases are the so-called consensus phytases of EP 98113176.6 (EP 0897985), viz.

(i) any thermostable phytase which is obtainable by the processes described therein;

(ii) a phytase comprising the amino acid sequence shown in Fig. 2 thereof or any variant or mutein thereof, preferred muteins being those comprising the substitutions Q50L; Q50T; Q50G; Q50T-Y51N or Q50L-Y51N.

Other preferred thermostable phytases are

(iii) a thermostable phytase which comprises at least one of the following amino acid sequence (some of which are shown in Figs. 5-12 herein), preferably the following phytases: Consensus-phytase-1 (or simply Consensus phytase); Consensus-phytase-1-thermo(3); Consensus-phytase-1-Q50T; basidiomycete-consensus (or simply Basidio); Consensus-phytase-10 (or Fcp 10); Consensus-phytase-11 (or Consensus Seq. 11); Consensus-phytase-1-thermo(8)-Q50T-K91A; Consensus-phytase-1-thermo(8)-Q50T; Consensus-phytase-1-thermo(8); Consensus-phytase-10-thermo(3)-Q50T-K91A; Consensus-phytase-10-thermo(3)-Q50T (sometimes, "(3)" is deleted from this expression); Aspergillus fumigatus ATCC 13073 phytase α-mutant; Aspergillus fumigatus ATCC 13073 phytase α-mutant plus the mutations E59A, S126N, R329H, S364T, G404A; Aspergillus fumigatus ATCC 13073 phytase α-mutant plus the mutations E59A, K68A, S126N, R329H, S364T, G404A; Consensus-phytase-7; Consensus-phytase-12.

(iv) as well as thermostable variants and muteins of the phytases of (iv) and (v), in particular those comprising one or more of the following substitutions: Q50L,T,G; Q50L-Y51N; Q50T-Y51N.

The term "plant" is intended to include not only whole plants as such, but also plant parts or organs, such as leaves, seeds or grains, stem, root, tubers, flowers, callus, fruits etc.; tissues, cells, protoplats etc.; as well as any combinations or sub-combinations thereof. Plant tissue cultures and plant cell lines as well as plant protoplasts are specifically included herein.

The term "transgenic plant" is a plant as defined above, which has been genetically modified, as well as its progeny and propagating material thereof having retained the genetical modification. Preferably, the transgenic plant comprises at least one specific gene introduced into an ancestral plant by recombinant gene technology. The term is not confined to a single plant variety.

The invention relates to a transgenic plant which comprises a DNA-construct encoding a thermostable phytase.

In a preferred embodiment the transgenic plant is a plant grouping which is characterized in that it comprises a DNA-construct encoding a thermostable phytase. The members of this plant grouping may very well possess individuality, but are clearly distinguishable from other varieties by their common characteristic feature of the the thermostable phytase DNA-construct.

Accordingly, the present teaching is applicable to more than one plant variety. No naturally occuring plant varieties are included amongst the plants of the invention.

In another preferred embodiment the invention relates to a transgenic plant variety or a variant thereof; a transgenic plant species, a transgenic plant genus, a transgenic plant family, and/or a transgenic plant order. More preferably, plant varieties as such are disclaimed.

Any thermostable phytase may be used in the present invention, e.g. any wild-type phytases, genetically engineered phytases, consensus phytases, phytase muteins, and/or phytase variants. Genetically engineered phytases include, but are not limited to, phytases prepared by site-directed mutagenesis, gene shuffling, random mutagenesis, etc.

The nucleotide sequence encoding a wild-type thermostable phytase may be of any origin, including mammalian, plant and microbial origin and may be isolated from these sources by conventional methods. Preferably, the nucleotide sequence is derived from a microorganism, such as a fungus, e.g. a yeast or a filamentous fungus, or a bacterium. The DNA sequence encoding a thermostable phytase may be isolated from the cell producing it, using various methods well known in the art (see e.g. WO 98/28409 and EP 0897985).

The nucleotide sequence encoding a thermostable genetically engineered or consensus phytase, including muteins and variants thereof, may be prepared in any way, e.g. as described in Example 3 hereof and in EP 0897985.

In order to accomplish expression of the thermostable phytase in a plant of the invention the nucleotide sequence encoding the phytase is inserted into an expression construct containing regulatory elements or sequences capable of directing the expression of the nucleotide sequence and, if necessary or desired, to direct secretion of the gene product or targetting of the gene product to the seeds of the plant.

In order for transcription to occur the nucleotide sequence encoding the thermostable phytase is operably linked to a suitable promoter capable of mediating transcription in the plant in question. The promoter may be an inducible promoter or a constitutive promoter. Typically, an inducible promoter mediates transcription in a tissue-specific or growth-stage specific manner, whereas a constitutive promoter provides for sustained transcription in all cell tissues. An example of a suitable constitutive promoter useful for the present invention is the cauliflower mosaic virus 35 S promoter. Transcription initiation sequences from the tumor-inducing plasmid (Ti) of

Agrobacterium such as the octopine synthase, nopaline synthase, or mannopine synthase initiator, are further examples of preferred constitutive promoters.

Examples of suitable inducible promoters include a seed-specific promoter such as the promoter expressing alpha-amylase in wheat seeds (see Stefanov et al, Acta Biologica Hungarica Vol. 42, No. 4 pp. 323-330 (1991), a promoter of the gene encoding a rice seed storage protein such as glutelin, prolamin, globulin or albumin (Wu et al., Plant and Cell Physiology Vol. 39, No. 8 pp. 885-889 (1998)), a Vicia faba promoter from the legumin B4 and the unknown seed protein gene from Vicia faba described by Conrad U. et al, Journal of Plant Physiology Vol. 152, No. 6 pp. 708-711 (1998), the storage protein napA promoter from Brassica napus, or any other seed specific promoter known in the art, eg as described in WO 91/14772.

In order to increase the expression of the thermostable phytase it is desirable that a promoter enhancer element is used. For instance, the promoter enhancer may be an intron which is placed between the promoter and the amylase gene. The intron may be one derived from a monocot or a dicot. For instance, the intron may be the first intron from the rice Waxy (Wx) gene (Li et al., Plant Science Vol. 108, No. 2, pp. 181-190 (1995)), the first intron from the maize Ubi1 (Ubiquitin) gene (Vain et al., Plant Cell Reports Vol. 15, No. 7 pp. 489-494 (1996)) or the first intron from the Act1 (actin) gene. As an example of a dicot intron the chsA intron (Vain et al. op cit.) is mentioned. Also, a seed specific enhancer may be used for increasing the expression of the thermostable phytase in seeds. An example of a seed specific enhancer is the one derived from the beta-phaseolin gene encoding the major seed storage protein of bean

(Phaseolus vulgaris) disclosed by Vandergeest and Hall, Plant Molecular Biology Vol. 32, No. 4, pp. 579-588 (1996).

Also, the expression construct preferably contains a terminator sequence to signal transcription termination of the thermostable phytase gene such as the rbcS2′ and the nos3′ terminators.

To facilitate selection of successfully transformed plants, the expression construct should also preferably include one or more selectable markers, e.g. an antibiotic resistance selection marker or a selection marker providing resistance to a herbicide. One widely used selection marker is the neomycin phosphotransferase gene (NPTII) which provides kanamycin resistance. Examples of other suitable markers include a marker providing a measurable enzyme activity, e.g. dihydrofolate reductase, luciferase, and b-glucoronidase (GUS). Phosphinothricin acetyl transferase may be used as a selection marker in combination with the herbicide basta or bialaphos.

The transgenic plant of the invention may be prepared by methods known in the art. The transformation method used will depend on the plant species to be transformed and can be selected from any of the transformation methods known in the art such as Agrobacterium mediated transformation (Zambryski et al., EMBO Journal 2, pp 2143-2150, 1993), particle bombardment, electroporation (Fromm et al. 1986, Nature 319, pp 791-793), and virus mediated transformation. For transformation of monocots particle bombardment (ie biolistic transformation) of embryogenic cell lines or cultured embryos are preferred. Below, references are listed, which disclose various methods for transforming various plants: Rice (Cristou et al. 1991, Bio/Technology 9, pp. 957-962), Maize (Gordon-Kamm et al. 1990, Plant Cell 2, pp. 603-618), Oat (Somers et al. 1992,

Bio/Technology 10, pp 1589-1594), Wheat (Vasil et al. 1991, Bio/Technology 10, pp. 667-674, Weeks et al. 1993, Plant Physiology 102, pp. 1077-1084) and Barley (Wan and Lemaux 1994, Plant Physiology 102, pp. 37-48, review Vasil 1994, Plant Mol. Biol. 25, pp 925-937).

More specifically, Agrobacterium mediated transformation is conveniently achieved as follows:

A vector system carrying the thermostable phytase is constructed. The vector system may comprise of one vector, but it can comprise of two vectors. In the case of two vectors the vector system is referred to as a binary vector system (Gynheung An et al.(1980), Binary Vectors, Plant Molecular Biology Manual A3, 1-19).

An Agrobacterium based plant transformation vector consists of replication origin(s) for both E.coli and Agrobacterium and a bacterial selection marker. A right and preferably also a left border from the Ti plasmid from Agrobacterium tumefaciens or from the Ri plasmid from Agrobacterium rhizogenes is nessesary for the transformation of the plant. Between the borders the expression construct is placed which contains the thermostable phytase gene and appropriate regulatory sequences such as promotor and terminator sequences. Additionally, a selection gene e.g. the neomycin phosphotransferase type II (NPTII) gene from transposon Tn5 and a reporter gene such as the GUS (betha-glucuronidase) gene is cloned between the borders. A disarmed Agrobacterium strain harboring a helper plasmid containing the virulens genes is transformed with the above vector. The transformed Agrobacterium strain is then used for plant transformation.

The invention also relates to a method of preparing a transgenic plant capable of expressing a thermostable phytase,

said method comprising the steps of (i) isolating a nucleotide sequence encoding a thermostable phytase; (ii) inserting the nucleotide sequence of (i) in an expression construct capable of mediating the expression of the nucleotide sequence in a selected host plant; and (iii) transforming the selected host plant with the expression construct.

The above method in which "at least one" replaces "a," when used in relation to the thermostable phytase, is also within this invention.

This method is an essentially non-biological method.

Any plant may be a selected host plant. More specifically, the plant can be dicotyledonous or monocotyledonous, for short a dicot or a monocot. Of primary interest are such plants which are potential food or feed components. These plants may comprise phytic acid. Examples of monocot plants are grasses, such as meadow grass (blue grass, Poa), forage grass such as festuca, lolium, temperate grass, such as Agrostis, and cereals, e.g. wheat, oats, rye, barley, rice, sorghum and maize (corn).

Examples of dicot plants are legumes, such as lupins, pea, bean and soybean, and cruciferous (family Brassicaceae), such as cauliflower, oil seed rape and the closely related model organism Arabidopsis thaliana.

Of particular interest are monocotyledonous plants, in particular crops or cereal plants such as wheat (Triticum, e.g. aestivum), barley (Hardeum, e.g. vulgare), oats, rye, rice, sorghum and corn (Zea, e.g. mays).

Of further particular interest are dicotyledonous plants, such as those mentioned above.

In a preferred embodiment, the ancestral plant or host plant is per se a desired feed ingredient.

## Examples

### Example 1

**FYT-assay - for analyzing phytase enzyme preparations**

The phytase activity can be measured using the following assay: 10 µl diluted enzyme samples (diluted in 0.1 M sodium acetate, 0.01 % Tween20, pH 5.5) are added into 250 µl 5 mM sodium phytate (Sigma) in 0.1 M sodium acetate, 0.01 % Tween20, pH 5.5 (pH adjusted after dissolving the sodium phytate; the substrate is preheated) and incubated for 30 minutes at 37°C. The reaction is stopped by adding 250 µl 10 % TCA and free phosphate is measured by adding 500 µl 7.3 g FeSO4 in 100 ml molybdate reagent (2.5 g $(NH_4)_6Mo_7O_{24}.4H_2O$ in 8 ml $H_2SO_4$ diluted to 250 ml). The absorbance at 750 nm is measured on 200 µl samples in 96 well microtiter plates. Substrate and enzyme blanks are included. A phosphate standard curve is also included (0-2 mM phosphate). 1 FYT equals the amount of enzyme that releases 1 µmol phosphate/min at the given conditions. This assay is preferred for phytase enzyme preparations (when not in admixture with other feed ingredients).

### Example 2

**FTU assay - for analyzing phytase in admixture with feed ingredients**

One FTU is defined as the amount of enzym, which at standard conditions (37°C, pH 5,5; reaction time 60 minutes and start concentration of phytic acid 5 mM) releases phosphate equivalent to 1 µmol phosphate per minute.

1 FTU = 1 FYT

The FTU assay is preferred for phytase activity measurements on animal feed premixes and the like complex compositions.

Reagents /substrates

Extraction buffer for feed etc.

This buffer is also used for preparation of $PO_4$-standards and further dilution of premix samples.

0,22 M acetate buffer with Tween 20 pH 5,5

30 g sodium acetate trihydrate (MW = 136,08 g/mol) e.g. Merck Art 46267 per liter and 0,1 g Tween 20 e.g. Merck Art 22184 pr. liter are weighed out.

The sodium acetate is dissolved in demineralised water.

Tween 20 is added, and pH adjusted to 5,50 ± 0,05 with acetic acid.

Add demineralised water to total volume.

Extraction buffer for premix

0,22 M acetate buffer with Tween 20, EDTA, $PO_4^{3-}$ og BSA.

30 g sodium acetate trihydrate e.g. Merck Art 6267 per liter.

0,1 g Tween 20 e.g. Merck Art 22184 per liter.

30 g EDTA f.eks. Merck Art 8418 pr. liter.

20 g $Na_2HPO_4$, $2H_2O$ e.g. Merck Art 6580 per liter.

0,5 g BSA (Bovine Serum Albumine, e.g. Sigma Art A-9647 per liter.

The ingredients are dissolved in demineralised water, and pH is adjusted to 5,50 ± 0,05 with acetic acid.

Add demineralised water to total volume.

BSA is not stable, and must therefore be added the same day the buffer is used.

50 mM $PO_4^{3-}$ stock solution

0,681 g KH2PO4 (MW = 136,09 g/mol) e.g. Merck Art 4873 is weighed out and dissolved in 100 ml 0,22 M sodium acetat with Tween, pH 5,5.

Storage stability: 1 week in refrigerator.

0,22 M acetate buffer pH 5,5 without Tween

This buffer is used for production of phytic acid substrate).

150 g sodium acetate trihydrate (MW = 136,08) e.g. Merck Art 6267 is weighed out and dissolved in demineralised water, and pH is adjusted with acetic acid to 5,50 ± 0,05.

Add demineralised water to 5000 ml.

Storage stability: 1 week at room temperature.

Phytic acid substrate; 5 mM phytic acid

The volume of phytic acid is calculated with allowance for the water content of the used batch.

If the water content is e.g. 8,4 % the following is obtained:

$$\frac{0{,}005\,mol/l \times 923{,}8\,g/mol}{(1 \div 0{,}084)} = 5{,}04\,g/l$$

Phytic acid (Na-salt) (MW = 923,8 g/mol) e.g. Sigma P-8810 is weighed out and dissolved in 0,22 M acetate buffer (without tween). Addition of (diluted) acetic acid increases the dissolution speed.

pH is adjusted to 5,50 ± 0,05 with acetic acid.

Add 0,22 M acetate buffer to total volume.

21,7 % nitric acid solution

For stop solution.

1 part concentrated (65%) nitric acid is mixed into 2 parts demineralised water.

Molybdate reagent

For stop solution.

100 g ammonium heptamolybdate tetrahydrate $(NH_4)6Mo_7O_{24},4H_2O$ e.g. Merck Art 1182 is dissolved in demineralised water. 10 ml 25 % $NH_3$ is added.

Add demineralised water to 1 liter.

0,24 % Ammonium vanadate

Bought from fra Bie & Berntsen.

Molybdat/vanadat stop solution

1 part vanadate solution (0,24 % ammonium vanadate) + 1 part molybdate solution are mixed. 2 parts 21,7 % nitric acid solution are added.

The solution is prepared not more than 2 hours before use, and the bottle is wrapped in tinfoil.

Samples

Frozen samples are defrosted in a refrigerator overnight.

Sample size for feed samples: At least 70 g, preferably 100 g.

Feed samples

Choose a solution volume which allows addition of buffer corresponding to 10 times the sample weight, e.g. 100 g is dissolved in 1000 ml 0,22 M acetate buffer with Tween, see enclosure 1. Round up to nearest solution volume.

If the sample size is approx. 100 g all the sample is ground in a coffee grinder and subsequently placed in tared

beakers. The sample weight is noted. It is not necessary to grind not-pelleted samples. If a sample is too big to handle, it is sample split into parts of approx. 100 g.

Magnets are placed in the beakers and 0,22 M acetate buffer with Tween is added.

The samples are extracted for 90 minutes.

After extraction the samples rest for 30 minuts to allow for the feed to sediment. A 5 ml sample is withdrawn with a pipette. The sample is taken 2 - 5 cm under the surface of the solution and placed in a centrifuge glass, which is covered by a lid.

The samples are centrifuged for 10 minutes at 4000 rpm.

Premix samples

Choose a solution volume which allows addition of buffer corresponding to 10 times the sample weight. Round up to nearest solution volume.

If the samples have been weighed (50 - 100 g) all of the sample is placed in tared beakers. The sample weight is noted. If a sample is too big to handle, it is split into parts of approx. 100 g.

Magnets are placed in the beakers and 0,22 M acetate buffer with Tween, EDTA og $PO_4^{3-}$ is added.

The samples are extracted for 60 minutes.

After extraction the samples rest for 30 minutes to allow for the premix to sediment. A 5 ml sample is withdrawn with a pipette. The sample is taken 2 - 5 cm under the surface of the solution and placed in a centrifuge glass, which is covered by a lid.

The samples are centrifuged for 10 minutes at 4000 rpm.

Analysis

Extracts of feed samples are analysed directly.

Extracts of premix are diluted to approx. 1,5 FTU/g ($A_{415}$ (main sample) < 1,0 ).

0,22 M acetate buffer with Tween 20 is used for the dilution.

Main Samples

2 x 100 ml of the supernatant from the extracted and centrifuged samples are placed in marked glass test tubes and a magnet is placed in each tube.

When all samples are ready they are placed on a water bath with stirring. Temperature: 37 °C.

3,0 ml substrate is added.

Incubation for exactly 60 minutes after addition of substrate.

The samples are taken off the water bath and 2,0 ml stop solution is added (exactly 60 minutes after addition of substrate).

The samples are stirred for 1 minute or longer.

Feed samples are centrifuged for 10 minutes at 4000 rpm (It is not necessary to centrifuge premix samples).

Blind samples

100 ml of the supernatant from the extracted and centrifuged samples are placed in marked glass test tubes, and a magnet is placed in each tube.

2,0 ml stop solution is added to the samples.

3,0 ml substrate is added to the samples.

The samples are incubated for 60 minutes at room temperature.

The feed samples are centrifuged for 10 minutes at 4000 rpm (it is not necessary to centrifuge premix samples).

Standards

2 x 100 ml are taken from each of the 8 standards and also 4 x 100 ml 0,22 M acetate buffer (reagent blind).

$A_{415}$ is measured on all samples.

CALCULATION

FTU/g = $\mu$mol $PO_4^{3-}$ / (min * g (sample))

*C* g sample is weighed out (after grinding).

100 $\mu$l is taken from the extracted and centrifuged sample.

$PO_4^{3-}$ standard curve is linear.

From the regression curve for the $PO_4^{3-}$ standard the actual concentration of the sample is found (concentration in mM):

$[PO_4^{3-}] = (x - b) / a$ $x = A_{415}$ *a = slope* *b = intercept with y-axis*

$\mu$mol $PO_4^{3-}$/min = { $[PO_4^{3-}]$ (mM) × Vol (liter) × 1000 $\mu$mol/mmol } /t

t = incubation time in minutes.

*Vol* = sample volume in liter = 0,0001 liter

1000 = conversion factor from mmol to $\mu$mol

$$FTU\ /g_{prøve} = \{ (x - b) \times Vol \times 1000 \times F_p\} / \{ a \times t \times C\}$$

$C$ = gram sample weighed out

$F_p$ = Relation between the sample taken out and the total sample (after extraction). Example: 0,100 ml taken from 1000 ml → $F_p$ = 1000/0,100 = 10000.

Reduced expression with insertion of the following values:

$t$ = 60

$Vol$ = 0,0001 l

$F_p$ = 10000

$$FTU\ /g_{sample} = \{ (x - b) \times 0{,}0001 \times 1000 \times 10000\} / \{ a \times 60 \times C\}$$

**Example 3**

**Determination of optimum temperature and melting point Tm of various phytases**

The thermostability of various phytases has been determined, viz. the melting temperature, Tm, and/or the optimum temperature.

The phytase of Aspergillus niger NRRL 3135 was prepared as described in EP 0420358 and van Hartingsveldt et al (Gene, 127, 87-94, 1993).

The phytases of Aspergillus fumigatus ATCC 13073, Aspergillus terreus 9A-1, Aspergillus terreus CBS 116.46, Aspergillus nidulans, Myceliophthora thermophila, and Talaromyces thermophilus were prepared as described in EP-0897985 and the references therein.

Consensus-phytase-1 (Fig. 5) and Consensus-phytase-1-Q50T are shown in and were prepared as described in EP 0897985.

Consensus-phytase-10 was derived and prepared according to the teachings of EP-0897985 (Examples 1-2 and 3-7, respectively), however adding to the alignment at Fig. 1 thereof the phytase sequence of Thermomyces lanuginosa (Berka et al, Appl. Environ. Microbiol. 64, 4423-4427, 1998) and a basidiomycete consensus sequence (derivation described below), omitting the sequence of A.niger T213, and assigning a vote weight of 0.5 for the remaining A.niger phytase sequences. The derivation of the sequence of Consensus-phytase-10 is shown in Fig. 7.

The basidiomycete consensus sequence was also derived according to the principles of EP-0897985, viz. from the five basidiomycete phytases of WO 98/28409, starting with the first amino acid residue of the mature phytases (excluding signal peptide). A vote weight of 0.5 was assigned to the two Paxillus phytases, all other genes were used with a vote weight of 1.0 - see Fig. 6.

The muteins Consensus-phytase-10-thermo, Consensus-phytase-10-thermo-Q50T-K91A (Fig. 10) and Consensus-phytase-10-thermo-Q50T were prepared from consensus-phytase-10, in analogy to Examples 5-8 of EP-0897985, by introducing the three back-mutations K94A, V158I and A396S ("thermo(3)" or "thermo") and, where applicable, also the mutations Q50T or Q50T-K91A.

The muteins Consensus-phytase-1-thermo(8), Consensus-phytase-1-thermo(8)-Q50T-K91A (Fig. 9) and Consensus-phytase-1-thermo(8)-Q50T, were prepared from consensus-phytase-1, in analogy to Example 8 of EP-0897985, by introducing the eight mutations E58A, D197N, E267D, R291I, R329H, S364T, A379K and G404A ("thermo(8)") and, where applicable, also the mutations Q50T or Q50T-K91A.

Consensus-phytase-1-thermo(3) was prepared from consensus-phytase-1 by introduction of the three mutations K94A, V158I and A396S.

An Aspergillus fumigatus so-called α-mutant (with the mutations Q51(27)T, F55Y, V100I, F114Y, A243L, S265P, N294D) and the further muteins thereof shown in Table 1 were prepared as generally described above. The position numbering refers to Fig. 11 hereof, except for the number in parentheses which refers to the numbering used in EP 0897010.

DNA constructs encoding the above thermostable phytases can be prepared e.g. according to the teachings of EP 0897985. For expression thereof in plants, reference is made to the present description.

In order to determine the unfolding temperature or melting temperature, Tm, of a phytase, differential scanning calorimetry was applied as previously published by Brugger et al (1997): "Thermal denaturation of phytases and pH 2.5 acid phosphatase studied by differential scanning calorimetry," in The Biochemistry of phytate and phytase (eds. Rasmussen, S.K; Raboy, V.; Dalbøge, H. and Loewus, F.; Kluwer Academic Publishers).

Homogenous or purified phytase solutions of 50-60 mg/ml of protein are prepared, and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10°C/min is applied up to 90-95°C.

The results of Tm determinations on the above phytases are shown in Table 1 below; for selected phytases also in Figs. 1-4.

In Table 1 below, the optimum temperature of various phytases is also indicated. For this determination, phytase activity was determined basically as described by Mitchell et al (Microbiology 143, 245-252, 1997): The activity was measured in an assay mixture containing 0.5% phytic acid (~ 5 mM) in 200 mM

sodium acetate, pH 5.0. After 15 min of incubation at 37°C, the reaction was stopped by addition of an equal volume of 15% trichloroacetic acid. The liberated phosphate was quantified by mixing 100 µl of the assay mixture with 900µl $H_2O$ and 1 ml of 0.6 M $H_2SO_4$, 2% ascorbic acid and 0.5% ammonium molybdate. Standard solutions of potassium phosphate were used as reference. One unit of enzyme activity was defined as the amount of enzyme that releases 1 µmol phosphate per minute at 37°C. The protein concentration was determined using the enzyme extinction coefficient at 280 nm calculated according to Pace et al (Prot.Sci. 4, 2411-2423, 1995): Consensus phytase, 1.101; consensus phytase 7, 1.068; consensus phytase 10, 1.039.

For determination of the temperature optimum, enzyme (100µl) and substrate solution (100µl) were pre-incubated for 5 min at the given temperature. The reaction was started by addition of the substrate solution to the enzyme. After 15 min incubation, the reaction was stopped with trichloroacetic acid and the amount of phosphate released was determined. Phytase-activity-versus-temperature is plotted, and the temperature optimum is determined as that temperature at which the acitivity reaches its maximum value.

Table 1

Temperature optimum and Tm for various phytases

| **Phytase** | **Optimum temperature (°C)** | **Tm (°C)** |
|---|---|---|
| Aspergillus niger NRRL 3135 | 55 | 63.3 |
| Aspergillus fumigatus ATCC 13073 | 55 | 62.5 |

| | | |
|---|---|---|
| Aspergillus terreus 9A-1 | 49 | 57.5 |
| Aspergillus terreus CBS 116.46 | 45 | 58.5 |
| Aspergillus nidulans | 45 | 55.7 |
| Myceliophthora thermophila | 55 | - |
| Talaromyces thermophilus | 45 | - |
| Consensus-phytase-10-thermo-Q50T-K91A | 82 | 89.3 |
| Consensus-phytase-10-thermo-Q50T | 82 | 88.6 |
| Consensus-phytase-10 | 80 | 85.4 |
| Consensus-phytase-1-thermo(8)-Q50T-K91A | - | 85.7 |
| Consensus-phytase-1-thermo(8)-Q50T | 78 | 84.7 |
| Consensus-phytase-1-thermo(8) | 81 | - |
| Consensus-phytase-1-thermo(8)-Q50T-K91A | 78 | 84.7 |
| Consensus-phytase-1-thermo(3) | 75 | - |
| Consensus-phytase-1-Q50T | - | 78.9 |
| Consensus-phytase-1 | 71 | 78.1 |
| Aspergillus fumigatus α-mutant, plus mutations E59A, | 63 | - |

| | | |
|---|---|---|
| S126N, R329H, S364T, G404A | | |
| Aspergillus fumigatus - as above, plus mutation K68A | 63 | - |
| Aspergillus fumigatus α-mutant (Q51(27)T, F55Y, V100I, F114Y, A243L, S265P, N294D) | 60 | 67.0 |

**CLAIMS**

1. A process of preparing an animal feed, which process comprises an agglomeration of feed ingredients, wherein a thermostable phytase is added before or during the agglomeration.

2. The process of claim 1, wherein the feed ingredients are heated to a temperature of at least 65°C.

3. The process of any of claims 1-2, wherein the thermostable phytase is a phytase with a Tm as measured by DSC of at least 65°C, using for the DSC a constant heating rate of 10°C/min.

4. The process of any of claims 1-3, when performed in a feed expander.

5. The process of any of claims 1-3, when performed in an extruder.

6. The process of any of claims 1-3, when performed in a pellet press.

7. The process of any of claims 1-6, wherein the thermostable phytase is present in a transgenic plant.

8. The process of any of claims 1-7, wherein the agglomeration includes the following steps:

(a) pre-heating the feed ingredients to a temperature of at least 45°C; and

(b) heating the product of step (a) to a temperature of at least 65°C;

wherein the thermostable phytase is added prior to or during step (a) and/or (b).

9. A transgenic plant which comprises a DNA-construct encoding a thermostable phytase.

10. The transgenic plant of claim 9, wherein the DNA-construct encoding the thermostable phytase is operably linked to regulatory sequences capable of mediating expression of said phytase encoding sequence in at least one part of the plant.

11. An expression construct which comprises a DNA construct encoding a thermostable phytase, operably linked to regulatory sequences capable of mediating expression of said phytase encoding sequence in at least one part of a plant.

12. A vector which comprises the expression construct of claim 11.

13. A method of preparing a transgenic plant capable of expressing a thermostable phytase, said method comprising the steps of

(i) isolating a nucleotide sequence encoding a thermostable phytase;

(ii) inserting the nucleotide sequence of (i) in an expression construct capable of mediating the expression of the nucleotide sequence in a selected host plant; and

(iii) transforming the selected host plant with the expression construct.

14. The method of claim 13, which comprises the further step of extracting the phytase from the plant.

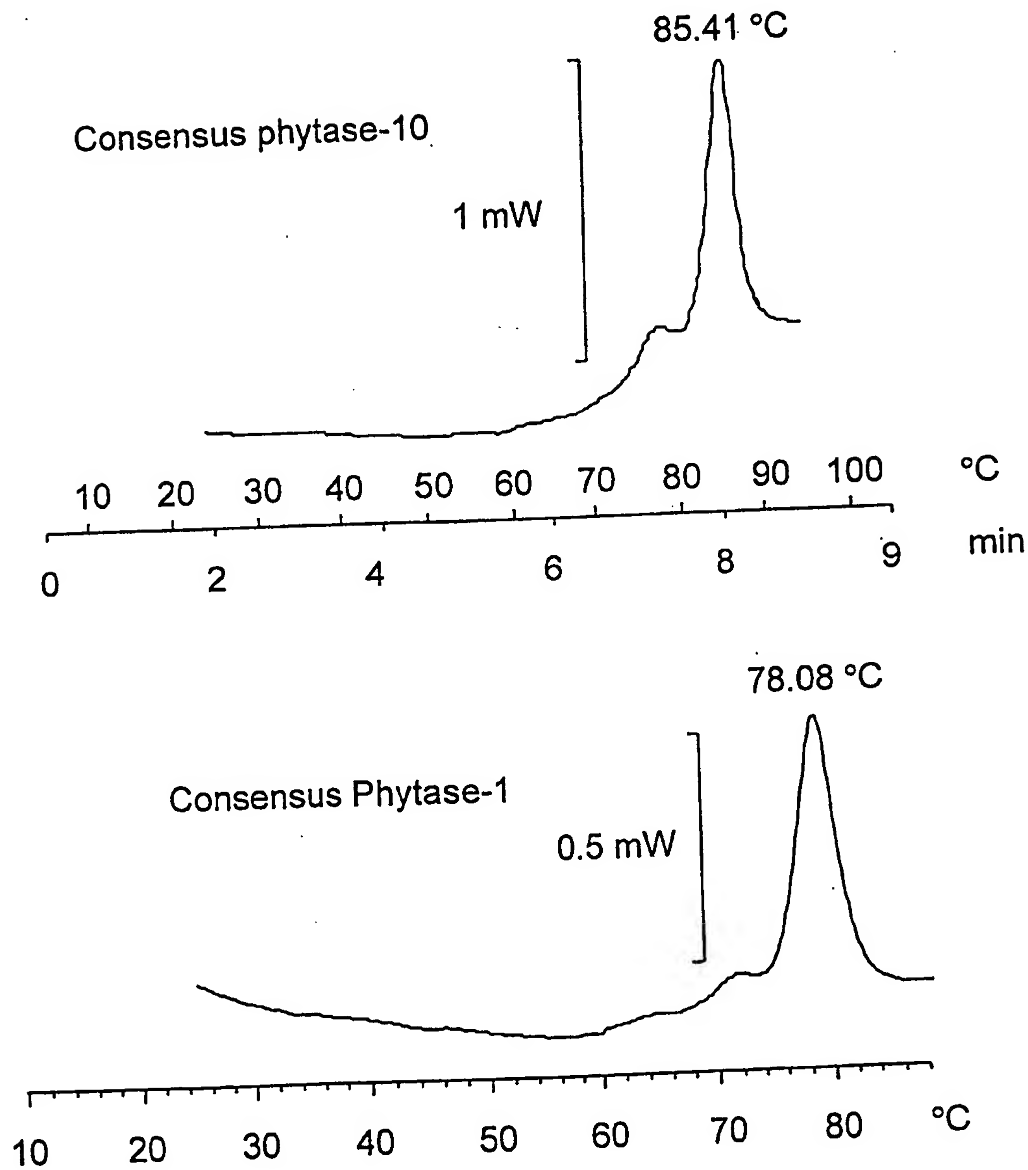

85.41 °C
Consensus phytase-10
1 mW
10 20 30 40 50 60 70 80 90 100 °C
0 2 4 6 8 9 min
78.08 °C
Consensus Phytase-1
0.5 mW
10 20 30 40 50 60 70 80 °C

Fig. 1

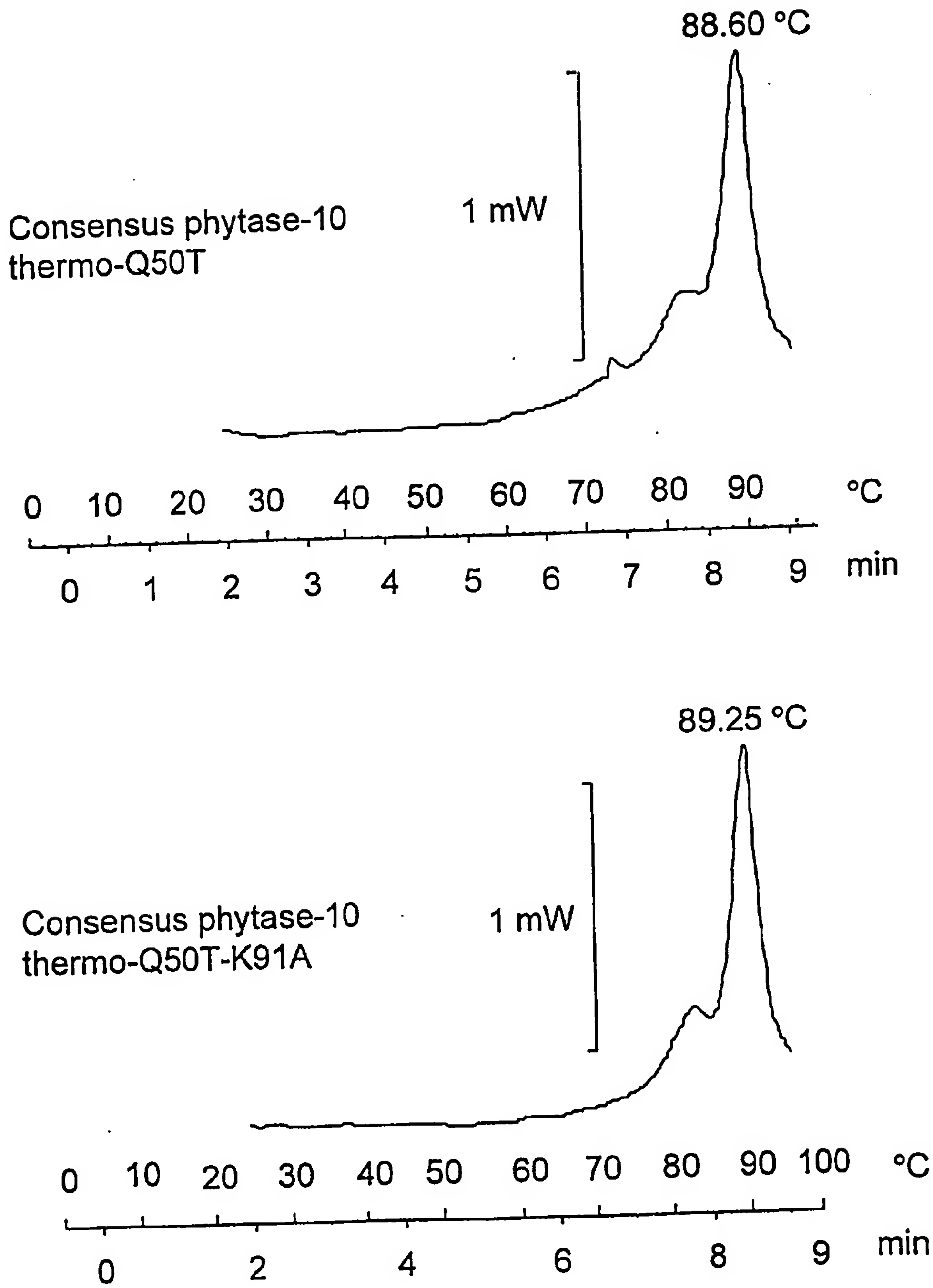
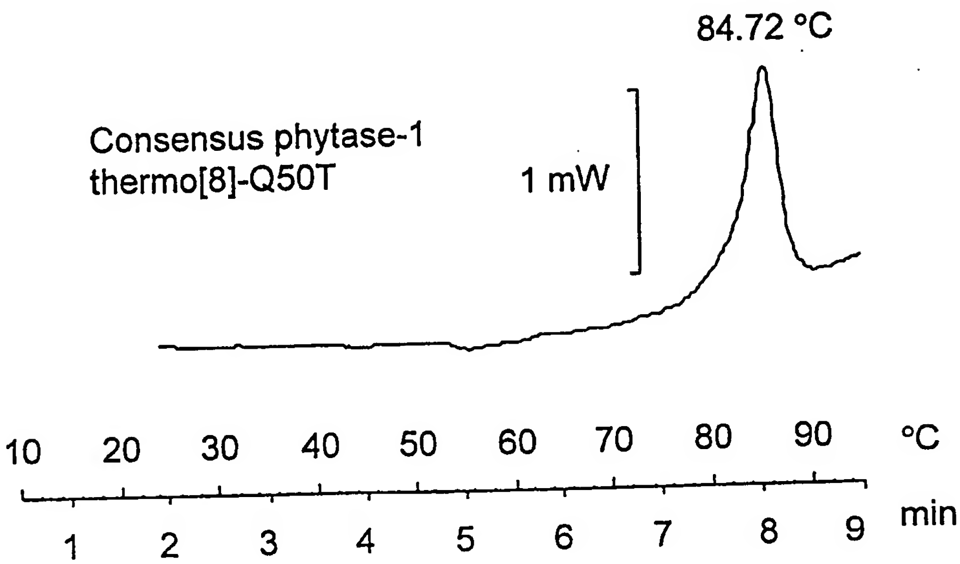

88.60 °C
1 mW
Consensus phytase-10
thermo-Q50T
0 10 20 30 40 50 60 70 80 90 °C
0 1 2 3 4 5 6 7 8 9 min
89.25 °C
1 mW
Consensus phytase-10
thermo-Q50T-K91A
0 10 20 30 40 50 60 70 80 90 100 °C
0 2 4 6 8 9 min

Fig. 2


84.72 °C
Consensus phytase-1
thermo[8]-Q50T
1 mW
10 20 30 40 50 60 70 80 90 °C
1 2 3 4 5 6 7 8 9 min

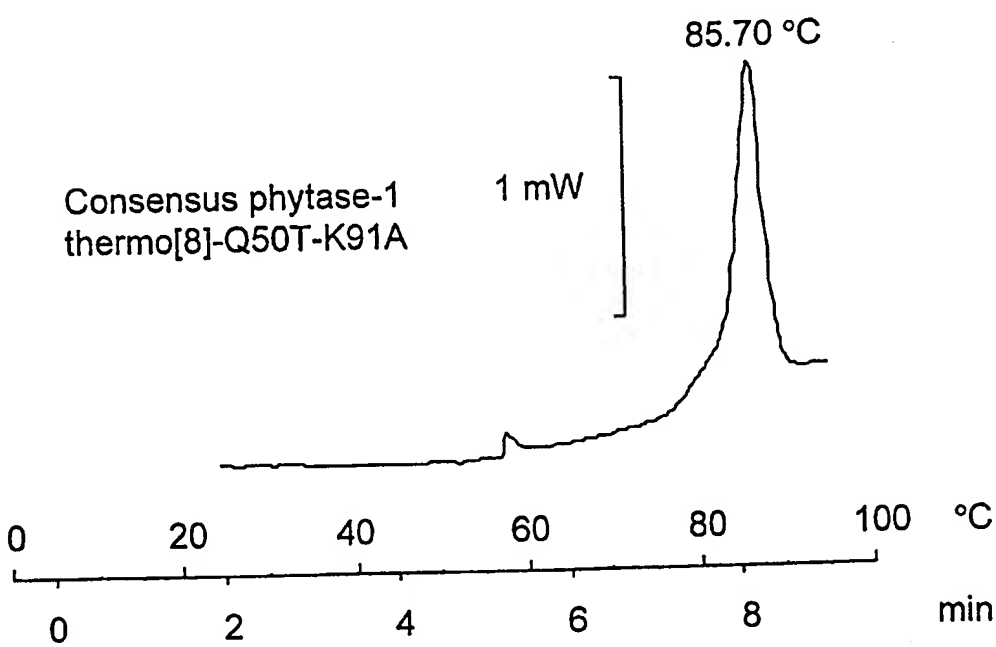
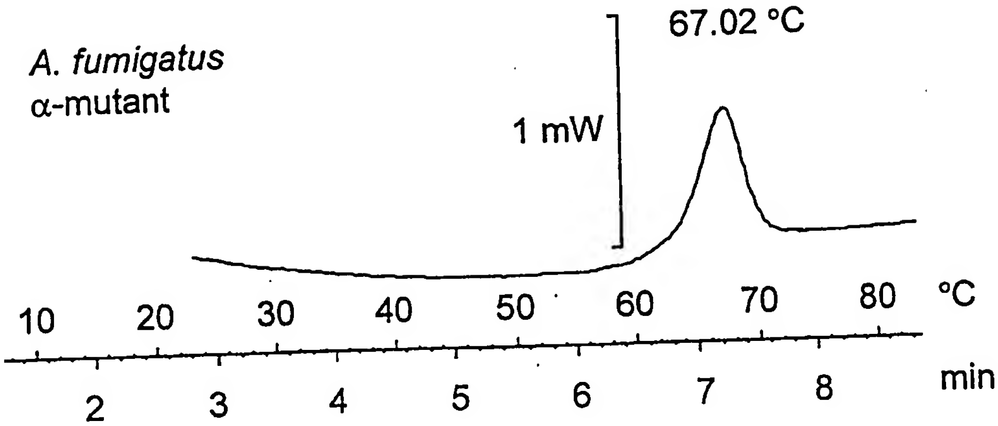
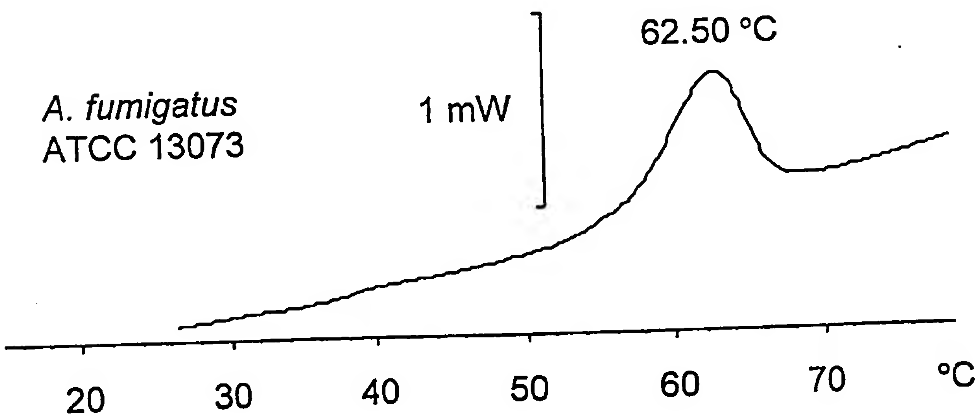

85.70 °C
Consensus phytase-1
thermo[8]-Q50T-K91A
1 mW
0 20 40 60 80 100 °C
0 2 4 6 8 min

Fig. 3



67.02 °C
A. fumigatus
α-mutant
1 mW
10
20
30
40
50
60
70
80
°C
2
3
4
5
6
7
8
min

62.50 °C
A. fumigatus
ATCC 13073
1 mW
20
30
40
50
60
70
°C


Fig. 4

```
                         1                                                    50
A. terreus 9A-1          KhsDCNSVDh GYQCFPELSH kWGlYAPYFS LQDESPFPlD VPEDChITFV
A. terreus cbs           NhsDCTSVDr GYQCFPELSH kWGlYAPYFS LQDESPFPlD VPDDChITFV
A. niger var. awamori    NqsTCDTVDQ GYQCFSETSH LWGQYAPFFS LANESAISPD VPAGCrVTFA
A. niger T213            NqsSCDTVDQ GYQCFSETSH LWGQYAPFFS LANESVISPD VPAGCrVTFA
A. niger NRRL3135        NqsSCDTVDQ GYQCFSETSH LWGQYAPFFS LANESVISPE VPAGCrVTFA
A. fumigatus 13073       GSkSCDTVDl GYQCsPATSH LWGQYSPFFS LEDElSVSSK LPKDCrITLV
A. fumigatus 32722       GSkSCDTVDl GYQCsPATSH LWGQYSPFFS LEDElSVSSK LPKDCrITLV
A. fumigatus 58128       GSkSCDTVDl GYQCsPATSH LWGQYSPFFS LEDElSVSSK LPKDCrITLV
A. fumigatus 26906       GSkSCDTVDl GYQCsPATSH LWGQYSPFFS LEDElSVSSK LPKDCrITLV
A. fumigatus 32239       GSkACDTVEl GYQCsPGTSH LWGQYSPFFS LEDElSVSSD LPKDCrVTFV
E. nidulans              QNHSCNTADG GYQCFPNVSH VWGQYSPYFS IEQESAISeD VPHGCeVTFV
T. thermophilus          DSHSCNTVEG GYQCrPEISH sWGQYSPFFS LADQSEISPD VPQNCkITFV
M. thermophila           ESRPCDTpDl GFQCgTAISH FWGQYSPYFS VpSElDaS.. IPDDCeVTFA

Consensus                NSHSCDTVDG GYQCFPEISH LWGQYSPYFS LEDESAISPD VPDDC-VTFV
Consensus phytase        NSHSCDTVDG GYQCFPEISH LWGQYSPYFS LEDESAISPD VPDDCRVTFV

                         51                                                   100
A. terreus 9A-1          QVLARHGARs PThSKtKAYA AtIAAIQKSA TaFpGKYAFL QSYNYSLDSE
A. terreus cbs           QVLARHGARs PTDSKtKAYA AtIAAIQKNA TaLpGKYAFL KSYNYSMGSE
A. niger var. awamori    QVLSRHGARY PTESKgKkYS ALIEEIQQNV TtFDGKYAFL KTYNYSLGAD
A. niger T213            QVLSRHGARY PTESKgKkYS ALIEEIQQNV TtFDGKYAFL KTYNYSLGAD
A. niger NRRL3135        QVLSRHGARY PTDSKgKkYS ALIEEIQQNA TtFDGKYAFL KTYNYSLGAD
A. fumigatus 13073       QVLSRHGARY PTSSKsKkYK kLVTAIQaNA TdFKGKFAFL KTYNYTLGAD
A. fumigatus 32722       QVLSRHGARY PTSSKsKkYK kLVTAIQaNA TdFKGKFAFL KTYNYTLGAD
A. fumigatus 58128       QVLSRHGARY PTSSKsKkYK kLVTAIQaNA TdFKGKFAFL KTYNYTLGAD
A. fumigatus 26906       QVLSRHGARY PTSSKsKkYK kLVTAIQaNA TdFKGKFAFL KTYNYTLGAD
A. fumigatus 32239       QVLSRHGARY PTASKsKkYK kLVTAIQKNA TeFKGKFAFL ETYNYTLGAD
E. nidulans              QVLSRHGARY PTESKsKAYS GLIEAIQKNA TsFwGQYAFL ESYNYTLGAD
T. thermophilus          QLLSRHGARY PTSSKtElYS QLISrIQKTA TaYKGyYAFL KDYrYqLGAN
M. thermophila           QVLSRHGARa PTlKRaaSYv DLIDrIHhGA IsYgPgYEFL RTYDYTLGAD

Consensus                QVLSRHGARY PTSSK-KAYS ALIEAIQKNA T-FKGKYAFL KTYNYTLGAD
Consensus phytase        QVLSRHGARY PTSSKSKAYS ALIEAIQKNA TAFKGKYAFL KTYNYTLGAD

                         101                                                  150
A. terreus 9A-1          ELTPFGrNQL rDlGaQFYeR YNALTRhInP FVRATDASRV hESAEKFVEG
A. terreus cbs           NLTPFGrNQL qDlGaQFYRR YDTLTRhInP FVRAADSSRV hESAEKFVEG
A. niger var. awamori    DLTPFGEQEL VNSGIKFYQR YESLTRNIIP FIRSSGSSRV IASGEKFIEG
A. niger T213            DLTPFGEQEL VNSGIKFYQR YESLTRNIIP FIRSSGSSRV IASGKKFIEG
A. niger NRRL3135        DLTPFGEQEL VNSGIKFYQR YESLTRNIVP FIRSSGSSRV IASGEKFIEG
A. fumigatus 13073       DLTPFGEQQL VNSGIKFYQR YKALARSVVP FIRASGSDRV IASGEKFIEG
A. fumigatus 32722       DLTPFGEQQL VNSGIKFYQR YKALARSVVP FIRASGSDRV IASGEKFIEG
A. fumigatus 58128       DLTPFGEQQL VNSGIKFYQR YKALARSVVP FIRASGSDRV IASGEKFIEG
A. fumigatus 26906       DLTAFGEQQL VNSGIKFYQR YKALARSVVP FIRASGSDRV IASGEKFIEG
A. fumigatus 32239       DLTPFGEQQM VNSGIKFYQK YKALAgSVVP FIRSSGSDRV IASGEKFIEG
E. nidulans              DLTiFGENQM VDSGaKFYRR YKNLARKnTP FIRASGSDRV VASAEKFING
T. thermophilus          DLTPFGENQM IQlGIKFYnH YKSLARNaVP FVRCSGSDRV IASGrlFIEG
M. thermophila           ELTRtGQQQM VNSGIKFYRR YRALARKsIP FVRTAGqDRV VhSAENFTQG

Consensus                DLTPFGENQM VNSGIKFYRR YKALARK-VP FVRASGSDRV IASAEKFIEG
Consensus phytase        DLTPFGENQM VNSGIKFYRR YKALARKIVP FIRASGSDRV IASAEKFIEG
```

Fig. 5A

| 151 – 200 | | | | | |
|---|---|---|---|---|---|
| *A. terreus* 9A-1 | FQTARqDDHh | ANpHQPSPrV | DVaIPEGSAY | NNTLEHSlCT | AFES...STV |
| *A. terreus* cbs | FQNARqGDPh | ANpHQPSPrV | DVVIPEGTAY | NNTLEHSICT | AFEA...STV |
| *A. niger* var. *awamori* | FQSTKLkDPr | AqpgQSSPkI | DVVISEASSs | NNTLDPGTCT | VFED...SEL |
| *A. niger* T213 | FQSTKLkDPr | AqpgQSSPkI | DVVISEASSs | NNTLDPGTCT | VFED...SEL |
| *A. niger* NRRL3135 | FQSTKLkDPr | AqpgQSSPkI | DVVISEASSs | NNTLDPGTCT | VFED...SEL |
| *A. fumigatus* 13073 | FQqAKLADPG | A.TNRAAPAI | SVIIPESETF | NNTLDHGVCT | kFEA...SQL |
| *A. fumigatus* 32722 | FQqAKLADPG | A.TNRAAPAI | SVIIPESETF | NNTLDHGVCT | kFEA...SQL |
| *A. fumigatus* 58128 | FQqAKLADPG | A.TNRAAPAI | SVIIPESETF | NNTLDHGVCT | kFEA...SQL |
| *A. fumigatus* 26906 | FQqAKLADPG | A.TNRAAPAI | SVIIPESETF | NNTLDHGVCT | kFEA...SQL |
| *A. fumigatus* 32239 | FQqANVADPG | A.TNRAAPVI | SVIIPESETY | NNTLDHSVCT | NFEA...SEL |
| *E. nidulans* | FRKAQLhDHG | S..gQATPVV | NVIIPEiDGF | NNTLDHSTCV | SFEN...DEr |
| *T. thermophilus* | FQSAKVlDPh | SDkHDAPPTI | NVIIeEGPSY | NNTLDtGSCP | VFED...SSg |
| *M. thermophila* | FHSAlLADRG | STvRPTlPyd | mVVIPETAGa | NNTLHNDlCT | AFEEgpySTI |
| Consensus | FQSAKLADPG | S-PHQASPVI | NVIIPEGSGY | NNTLDHGTCT | AFED---SEL |
| Consensus phytase | FQSAKLADPG | SQPHQASPVI | DVIIPEGSGY | NNTLDHGTCT | AFED...SEL |

| 201 – 250 | | | | | |
|---|---|---|---|---|---|
| *A. terreus* 9A-1 | GDDAvANFTA | VFAPAIaQRL | EADLPGVqLS | TDDVVnLMAM | CPFETVSlTD |
| *A. terreus* cbs | GDAAADNFTA | VFAPAIakRL | EADLPGVqLS | ADDVVnLMAM | CPFETVSlTD |
| *A. niger* var. *awamori* | ADTVEANFTA | TFAPSIRQRL | ENDLSGVTLT | DTEVTyLMDM | CSFDTISTST |
| *A. niger* T213 | ADTVEANFTA | TFAPSIRQRL | ENDLSGVTLT | DTEVTyLMDM | CSFDTIStST |
| *A. niger* NRRL3135 | ADTVEANFTA | TFVPSIRQRL | ENDLSGVTLT | DTEVTyLMDM | CSFDTIStST |
| *A. fumigatus* 13073 | GDEVAANFTA | lFAPDIRARa | EkHLPGVTLT | DEDVVsLMDM | CSFDTVARTS |
| *A. fumigatus* 32722 | GDEVAANFTA | lFAPDIRARa | EkHLPGVTLT | DEDVVsLMDM | CSFDTVARTS |
| *A. fumigatus* 58128 | GDEVAANFTA | lFAPDIRARa | EkHLPGVTLT | DEDVVsLMDM | CSFDTVARTS |
| *A. fumigatus* 26906 | GDEVAANFTA | lFAPDIRARa | KkHLPGVTLT | DEDVVsLMDM | CSFDTVARTS |
| *A. fumigatus* 32239 | GDEVEANFTA | lFAPAIRARI | EkHLPGVqLT | DDDVVsLMDM | CSFDTVARTA |
| *E. nidulans* | ADEiEANFTA | IMGPPIRkRL | ENDLPGIKLT | NENVIyLMDM | CSFDTMARTA |
| *T. thermophilus* | GHDAQEKFAk | qFAPAIlEKI | KDHLPGVDLA | vSDVpyLMDL | CPFETLARNh |
| *M. thermophila* | GDDAQDTYlS | TFAGPItARV | NANLPGANLT | DADTVaLMDL | CPFETVAsSS |
| Consensus | GDDAEANFTA | TFAPAIRARL | EADLPGVTLT | DEDVV-LMDM | CPFETVARTS |
| Consensus phytase | GDDVEANFTA | LFAPAIRARL | EADLPGVTLT | DEDVVYLMDM | CPFETVARTS |

| 251 – 300 | | | | | |
|---|---|---|---|---|---|
| *A. terreus* 9A-1 | ........ | ...DAhTLSPFC | DLFTAtEWtq | YNYLlSLDKY | YGYGGGNPLG |
| *A. terreus* cbs | ........ | ...DAhTLSPFC | DLFTAaEWtq | YNYLlSLDKY | YGYGGGNPLG |
| *A. niger* var. *awamori* | ........ | ...vDTKLSPFC | DLFTHdEWih | YDYLQSLkKY | YGHGAGNPLG |
| *A. niger* T213 | ........ | ...vDTKLSPFC | DLFTHdEWih | YDYLRSLkKY | YGHGAGNPLG |
| *A. niger* NRRL3135 | ........ | ...vDTKLSPFC | DLFTHdEWin | YDYLQSLkKY | YGHGAGNPLG |
| *A. fumigatus* 13073 | ........ | ...DASQLSPFC | QLFTHnEWkk | YNYLQSLGKY | YGYGAGNPLG |
| *A. fumigatus* 32722 | ........ | ...DASQLSPFC | QLFTHnEWkk | YNYLQSLGKY | YGYGAGNPLG |
| *A. fumigatus* 58128 | ........ | ...DASQLSPFC | QLFTHnEWkk | YNYLQSLGKY | YGYGAGNPLG |
| *A. fumigatus* 26906 | ........ | ...DASQLSPFC | QLFTHnEWkk | YNYLQSLGKY | YGYGAGNPLG |
| *A. fumigatus* 32239 | ........ | ...DASELSPFC | AIFTHnEWkk | YDYLQSLGKY | YGYGAGNPLG |
| *E. nidulans* | ........ | ...HGTELSPFC | AIFTEkEWlq | YDYLQSLSKY | YGYGAGSPLG |
| *T. thermophilus* | ........ | ...TDT.LSPFC | ALsTQeEWqa | YDYYQSLGKY | YGnGGGNPLG |
| *M. thermophila* | sdpatadagg | gNGrpLSPFC | rLFSEsEWra | YDYLQSVGKW | YGYGPGNPLG |
| Consensus | ---------- | -DATELSPFC | ALFTE-EW-- | YDYLQSLGKY | YGYGAGNPLG |
| Consensus phytase | .......... | .DATELSPFC | ALFTHDEWRQ | YDYLQSLGKY | YGYGAGNPLG |

Fig. 5B

```
                          301                                                    350
A. terreus 9A-1           PVQGVGWaNE LMARLTRAPV HDHTCVNNTL DASPATFPLN ATLYADFSHD
A. terreus cbs            PVQGVGWaNE LIARLTRSPV HDHTCVNNTL DANPATFPLN ATLYADFSHD
A. niger var. awamori     PTQGVGYaNE LIARLTHSPV HDDTSSNHTL DSNPATFPLN STLYADFSHD
A. niger T213             PTQGVGYaNE LIARLTHSPV HDDTSSNHTL DSNPATFPLN STLYADFSHD
A. niger NRRL3135         PTQGVGYaNE LIARLTHSPV HDDTSSNHTL DSSPATFPLN STLYADFSHD
A. fumigatus 13073        PAQGIGFtNE LIARLTRSPV QDHTSTNsTL vSNPATFPLN ATMYVDFSHD
A. fumigatus 32722        PAQGIGFtNE LIARLTRSPV QDHTSTNsTL vSNPATFPLN ATMYVDFSHD
A. fumigatus 58128        PAQGIGFtNE LIARLTRSPV QDHTSTNsTL vSNPATFPLN ATMYVDFSHD
A. fumigatus 26906        PAQGIGFtNE LIARLTRSPV QDHTSTNsTL vSNPATFPLN ATMYVDFSHD
A. fumigatus 32239        PAQGIGFtNE LIARLTNSPV QDHTSTNsTL DSDPATFPLN ATIYVDFSHD
E. nidulans               PAQGIGFtNE LIARLTQSPV QDNTSTNHTL DSNPATFPLD rKLYADFSHD
T. thermophilus           PAQGVGFvNE LIARMTHSPV QDYTTVNHTL DSNPATFPLN ATLYADFSHD
M. thermophila            PTQGVGFvNE LLARLAgvPV RDgTSTNRTL DGDPrTFPLG rPLYADFSHD

Consensus                 PAQGVGF-NE LIARLTHSPV QDHTSTNHTL DSNPATFPLN ATLYADFSHD
Consensus phytase         PAQGVGFANE LIARLTRSPV QDHTSTNHTL DSNPATFPLN ATLYADFSHD

                          351                                                    400
A. terreus 9A-1           SNLVSIFWAL GLYNGTAPLS qTSVESVSQT DGYAAAWTVP FAARAYVEMM
A. terreus cbs            SNLVSIFWAL GLYNGTkPLS qTTVEDITrT DGYAAAWTVP FAARAYIEMM
A. niger var. awamori     NGIISILFAL GLYNGTkPLS TTTVENITQT DGFSSAWTVP FASRlYVEMM
A. niger T213             NGIISILFAL GLYNGTkPLS TTTVENITQT DGFSSAWTVP FASRlYVEMM
A. niger NRRL3135         NGIISILFAL GLYNGTkPLS TTTVENITQT DGFSSAWTVP FASRlYVEMM
A. fumigatus 13073        NSMVSIFFAL GLYNGTEPLS rTSVESaKEl DGYSASWVVP FGARAYFEtM
A. fumigatus 32722        NSMVSIFFAL GLYNGTGPLS rTSVESaKEl DGYSASWVVP FGARAYFEtM
A. fumigatus 58128        NSMVSIFFAL GLYNGTEPLS rTSVESaKEl DGYSASWVVP FGARAYFEtM
A. fumigatus 26906        NSMVSIFFAL GLYNGTEPLS rTSVESaKEl DGYSASWVVP FGARAYFEtM
A. fumigatus 32239        NGMIPIFFAM GLYNGTEPLS qTSeESTKES NGYSASWAVP FGARAYFEtM
E. nidulans               NSMISIFFAM GLYNGTQPLS mDSVESIQEm DGYAASWTVP FGARAYFELM
T. thermophilus           NTMTSIFaAL GLYNGTAkLS TTEIKSIEET DGYSAAWTVP FGGRAYIEMM
M. thermophila            NDMMGVLgAL GaYDGVPPLD KTArrDpEEl GGYAASWAVP FAARiYVEKM

Consensus                 NSMISIFFAL GLYNGTAPLS TTSVESIEET DGYAASWTVP FGARAYVEMM
Consensus phytase         NSMISIFFAL GLYNGTAPLS TTSVESIEET DGYSASWTVP FGARAYVEMM

                          401                                                    450
A. terreus 9A-1           QC........ .....RAEKE PLVRVLVNDR VMPLHGCPTD KLGRCKrDAF
A. terreus cbs            QC........ .....RAEKQ PLVRVLVNDR VMPLHGCAVD NLGRCKrDDF
A. niger var. awamori     QC........ .....QAEQE PLVRVLVNDR VVPLHGCPID aLGRCTrDSF
A. niger T213             QC........ .....QAEQE PLVRVLVNDR VVPLHGCPID aLGRCTrDSF
A. niger NRRL3135         QC........ .....QAEQE PLVRVLVNDR VVPLHGCPVD aLGRCTrDSF
A. fumigatus 13073        QC........ .....KSEKE PLVRALINDR VVPLHGCDVD KLGRCKLNDF
A. fumigatus 32722        QC........ .....KSEKE PLVRALINDR VVPLHGCDVD KLGRCKLNDF
A. fumigatus 58128        QC........ .....KSEKE SLVRALINDR VVPLHGCDVD KLGRCKLNDF
A. fumigatus 26906        QC........ .....KSEKE PLVRALINDR VVPLHGCDVD KLGRCKLNDF
A. fumigatus 32239        QC........ .....KSEKE PLVRALINDR VVPLHGCAVD KLGRCKLKDF
E. nidulans               QC........ .....E.KKE PLVRVLVNDR VVPLHGCAVD KFGRCTLDDW
T. thermophilus           QC........ .....DDSDE PVVRVLVNDR VVPLHGCEVD SLGRCKrDDF
M. thermophila            RCsggggggg ggegrQEKDE eMVRVLVNDR VMTLkGCGAD ErGMCTLErF

Consensus                 QC-------- -----QAEKE PLVRVLVNDR VVPLHGCAVD KLGRCKLDDF
Consensus phytase         QC........ .....QAEKE PLVRVLVNDR VVPLHGCAVD KLGRCKRDDF

                          451                   471
A. terreus 9A-1           VAGLSFAQAG GNWADCF--- -
A. terreus cbs            VEGLSFARAG GNWAECF--- -
A. niger var. awamori     VrGLSFARSG GDWAECsA-- -
A. niger T213             VrGLSFARSG GDWAECFA-- -
A. niger NRRL3135         VrGLSFARSG GDWAECFA-- -
A. fumigatus 13073        VKGLSWARSG GNWGECFS-- -
A. fumigatus 32722        VKGLSWARSG GNWGECFS-- -
A. fumigatus 58128        VKGLSWARSG GNWGECFS-- -
A. fumigatus 26906        VKGLSWARSG GNWGECFS-- -
A. fumigatus 32239        VKGLSWARSG GNSEQSFS-- -
E. nidulans               VEGLNFARSG GNWkTCFTl- -
```

Fig. 5C

| | |
|---|---|
| *T. thermophilus* | VrGLSFARqG GNWEGCYAas e |
| *M. thermophila* | IESMAFARGN GKWDlCFA-- - |
| | |
| Consensus | VEGLSFARSG GNWAECFA-- - |
| Consensus phytase | VEGLSFARSG GNWAECFA.. . |

Fig. 5D

```
                                                                                  50
                         1
P. involutus (phyA1)  SvP.KnTAPt FPIPeseQrn WSPYSPYFPL AeYkAPPAGC QInQVNIIQR
P. involutus (phyA2)  SvP.RniAPK FSIPeseQrn WSPYSPYFPL AeYkAPPAGC EInQVNIIQR
T. pubescens          hiPlRdTSAc LdVTrDvQqs WSmYSPYFPa AtYvAPPASC QInQVHIIQR
A. pediades           GgvvQaTfvQ pfFPpQiQds WAAYTPYYPV qaYtPPPkDC KItQVNIIQR
P. lycii              StQfsfvAAQ LPIPaQntsn WGPYdPFFPV EpYaAPPEGC tVtQVNLIQR

Basidio               S-P-R-TAAQ LPIP-Q-Q-- WSPYSPYFPV A-Y-APPAGC QI-QVNIIQR

                                                                                 100
                         51
P. involutus (phyA1)  HGARFPTSGA TTRIKAGLTK LQGvqnfTDA KFNFIkSfkY dLGnsDLVPF
P. involutus (phyA2)  HGARFPTSGA ATRIKAGLSK LQSvqnfTDP KFDFIkSfTY dLGtsDLVPF
T. pubescens          HGARFPTSGA AkRIQTAVAK LKAAsnyTDP lLAFVtNyTY sLGqDsLVeL
A. pediades           HGARFPTSGA GTRIQAAVkK LQSAktyTDP RLDFLtNyTY tLGhDDLVPF
P. lycii              HGARWPTSGA rSRqvAAVAK IQmArpfTDP KYEFLnDfvY kFGvADLLPF

Basidio               HGARFPTSGA ATRIQAAVAK LQSA---TDP KLDFL-N-TY -LG-DDLVPF

                                                                                 150
                         101
P. involutus (phyA1)  GAaQSfDAGQ EAFARYSkLV SkNNLPFIRA dGSDRVVDSA TNWTAGFAsA
P. involutus (phyA2)  GAaQSfDAGl EvFARYSkLV SsDNLPFIRS dGSDRVVDTA TNWTAGFAsA
T. pubescens          GAtQSSEAGQ EAFTRYSsLV SaDELPFVRA SGSDRVVATA nNWTAGFAlA
A. pediades           GAlQSSQAGE ETFqRYSfLV SkENLPFVRA SSSNRVVDSA TNWTEGFSaA
P. lycii              GAnQShQTGt DmYTRYStLf egGDVPFVRA AGdQRVVDSS TNWTAGFGdA

Basidio               GA-QSSQAGQ EAFTRYS-LV S-DNLPFVRA SGSDRVVDSA TNWTAGFA-A

                                                                                 200
                         151
P. involutus (phyA1)  ShNTvqPkLn LILPQtGNDT LEDNMCPaAG DSDPQvNaWL AVafPSITAR
P. involutus (phyA2)  SrNAiqPkLd LILPQtGNDT LEDNMCPaAG ESDPQvDaWL AsafPSVTAQ
T. pubescens          SsNSitPvLs VIISEaGNDT LDDNMCPaAG DSDPQvNqWL AqFAPPMTAR
A. pediades           ShHvlnPiLf VILSEslNDT LDDaMCPnAG sSDPQtGiWt SIYGTPIAnR
P. lycii              SgETvlPtLq VVLqEeGNcT LcNNMCPnEv DGDest.tWL GVFAPnITAR

Basidio               S-NT--P-L- VILSE-GNDT LDDNMCP-AG DSDPQ-N-WL AVFAPPITAR

                                                                                 250
                         201
P. involutus (phyA1)  LNAAAPSvNL TDtDAfNLvs LCAFlTVSkE kkSdFCtLFE giPGsFeAFa
P. involutus (phyA2)  LNAAAPGANL TDaDAfNLvs LCPFmTVSkE qkSdFCtLFE giPGsFeAFa
T. pubescens          LNAGAPGANL TDtDTyNLlt LCPFETVAtE rrSeFCDIYE elQAE.dAFa
A. pediades           LNqqAPGANI TAaDvsNLip LCAFETIvkE tpSpFCNLF. .tPEEFaqFe
P. lycii              LNAAAPSANL SDsDAltLmd MCPFDTLSsG naSpFCDLF. .tAEEYvSYe

Basidio               LNAAAPGANL TD-DA-NL-- LCPFETVS-E --S-FCDLFE --PEEF-AF-

                                                                                 300
                         251
P. involutus (phyA1)  YgGDLDKFYG TGYGQeLGPV QGVGYVNELI ARLTnsAVRD NTQTNRTLDA
P. involutus (phyA2)  YaGDLDKFYG TGYGQALGPV QGVGYINELL ARLTnsAVnD NTQTNRTLDA
T. pubescens          YnADLDKFYG TGYGQPLGPV QGVGYINELI ARLTaQnVsD HTQTNsTLDS
A. pediades           YfGDLDKFYG TGYGQPLGPV QGVGYINELL ARLTemPVRD NTQTNRTLDS
P. lycii              YyyDLDKYYG TGpGNALGPV QGVGYVNELL ARLTgQAVRD ETQTNRTLDS

Basidio               Y-GDLDKFYG TGYGQPLGPV QGVGYINELL ARLT-QAVRD NTQTNRTLDS

                                                                                 350
                         301
```

Fig. 6A

```
P. involutus (phyA1) SPvTFPLNKT FYADFSHDNl MVAVFSAMGL FrQPAPLsTS vPNPwRTWrT
P. involutus (phyA2) APdTFPLNKT MYADFSHDNl MVAVFSAMGL FrQSAPLsTS tPDPNRTWLT
T. pubescens         SPeTFPLNRT LYADFSHDNQ MVAIFSAMGL FNQSAPLDPT tPDPaRTFLv
A. pediades          SPlTFPLDRS IYADLSHDNQ MIAIFSAMGL FNQSSPLDPS fPNPKRTWVT
P. lycii             dPaTFPLNRT FYADFSHDNt MVPIFAALGL FNaTA.LDPl kPDeNRlWVd

Basidio              SP-TFPLNRT FYADFSHDNQ MVAIFSAMGL FNQSAPLDPS -PDPNRTWVT

                     351                                             400
P. involutus (phyA1) SsLVPFSGRM VVERLsC..f GT........ .......tkV RVLVQDqVQP
P. involutus (phyA2) SsVVPFSARM aVERLsC..a GT........ .......tkV RVLVQDqVQP
T. pubescens         kKIVPFSARM VVERLdC..g GA........ .......qsV RLLVNDAVQP
A. pediades          SRLtPFSARM VtERLlCqrd GTgsggpsri mrngnvqtfV RILVNDALQP
P. lycii             SKLVPFSGHM tVEKLaC... .......... ....sgkeaV RVLVNDAVQP

Basidio              SKLVPFSARM VVERL-C--- GT-------- ---------V RVLVNDAVQP

                     401                                     441
P. involutus (phyA1) LEFCGGDrNG lCTLAkFVES QtFARsDGaG DFEKCFATSa ~
P. involutus (phyA2) LEFCGGDqDG lCALDkFVES QaYARsGGaG DFEKCLATTv ~
T. pubescens         LAFCGADtsG vCTLDAFVES QaYARNDGEG DFEKCFAT~~ ~
A. pediades          LKFCGGDmDS lCTLEAFVES QkYAREDGQG DFEKCFD~~~ ~
P. lycii             LEFCGG.vDG vCeLsAFVES QtYARENGQG DFAKCgfvPs e

Basidio              LEFCGGD-DG -CTLDAFVES Q-YAREDGQG DFEKCFATP- -
```

Fig. 6B

| | | | | | |
|---|---|---|---|---|---|
| | 1 | | | | 50 |
| *A. terreus* 9a1 | KhsdCNSVDh | GYQCfPELSH | kWGlYAPYFS | LqDESPFPlD | VPeDCHITFV |
| *A. terreus* cbs | NhsdCtSVDr | GYQCfPELSH | kWGlYAPYFS | LqDESPFPlD | VPdDCHITFV |
| *A. niger* var. *awamori* | NqsTCDTVDq | GYQCfSEtSH | LWGQYAPFFS | LANESAISPD | VPaGCRVTFa |
| *A. niger* NRRL3135 | NqsSCDTVDq | GYQCfSEtSH | LWGQYAPFFS | LANESvISPE | VPaGCRVTFa |
| *A. fumigatus* 13073 | GSkSCDTVDl | GYQCsPAtSH | LWGQYSPFFS | LEDElSVSSK | LPkDCRITLV |
| *A. fumigatus* 32722 | GSkSCDTVDl | GYQCsPAtSH | LWGQYSPFFS | LEDElSVSSK | LPkDCRITLV |
| *A. fumigatus* 58128 | GSkSCDTVDl | GYQCsPAtSH | LWGQYSPFFS | LEDElSVSSK | LPkDCRITLV |
| *A. fumigatus* 26906 | GSkSCDTVDl | GYQCsPAtSH | LWGQYSPFFS | LEDElSVSSK | LPkDCRITLV |
| *A. fumigatus* 32239 | GSkACDTVEl | GYQCsPGtSH | LWGQYSPFFS | LEDElSVSSD | LPkDCRVTFV |
| *E. nidulans* | QNHSCNTaDG | GYQCfPNVSH | VWGQYSPYFS | IEQESAISeD | VPhGCeVTFV |
| *T. thermophilus* | DSHSCNTVEG | GYQCrPEISH | sWGQYSPFFS | LADQSEISPD | VPqNCKITFV |
| *T. lanuginosa* | ---------- | ----nvDIAR | hWGQYSPFFS | LAEvSEISPA | VPkGCRVeFV |
| *M. thermophila* | ESRPCDTpDl | GFQCgTAISH | FWGQYSPYFS | VPsElDaS.. | IPdDCeVTFa |
| Basidio | xSxPxrxtAA | qLPipxQxqx | xWSPYSPYFP | VAxyxA.... | pPaGCQIxqV |
| Consensus | NSHSCDTVDG | GYQC-PEISH | LWGQYSPFFS | LADESAISPD | VP-GCRVTFV |
| Fcp10 | NSHSCDTVDG | GYQCFPEISH | LWGQYSPFFS | LADESAISPD | VPKGCRVTFV |

| | | | | | |
|---|---|---|---|---|---|
| | 51 | | | | 100 |
| *A. terreus* 9a1 | QVLARHGARs | PThSKTKaYA | AtIaAIQKSA | TaFpGKYAFL | QSYNYSLDSE |
| *A. terreus* cbs | QVLARHGARs | PTdSKTKaYA | AtIaAIQKNA | TaLpGKYAFL | KSYNYSMGSE |
| *A. niger* var. *awamori* | QVLSRHGARY | PTeSKGKKYS | ALIeEIQQNv | TtFDGKYAFL | KTYNYSLGAD |
| *A. niger* NRRL3135 | QVLSRHGARY | PTdSKGKKYS | ALIeEIQQNA | TtFDGKYAFL | KTYNYSLGAD |
| *A. fumigatus* 13073 | QVLSRHGARY | PTSSKSKKYk | kLVtAIQaNA | TdFKGKFAFL | KTYNYTLGAD |
| *A. fumigatus* 32722 | QVLSRHGARY | PTSSKSKKYk | kLVtAIQaNA | TdFKGKFAFL | KTYNYTLGAD |
| *A. fumigatus* 58128 | QVLSRHGARY | PTSSKSKKYk | kLVtAIQaNA | TdFKGKFAFL | KTYNYTLGAD |
| *A. fumigatus* 26906 | QVLSRHGARY | PTSSKSKKYk | kLVtAIQaNA | TdFKGKFAFL | KTYNYTLGAD |
| *A. fumigatus* 32239 | QVLSRHGARY | PTASKSKKYk | kLVtAIQKNA | TeFKGKFAFL | ETYNYTLGAD |
| *E. nidulans* | QVLSRHGARY | PTeSKSKaYS | GLIeAIQKNA | TsFwGQYAFL | ESYNYTLGAD |
| *T. thermophilus* | QLLSRHGARY | PTSSKTElYS | qLIsrIQKtA | TaYKGyYAFL | KdYrYqLGAN |
| *T. lanuginosa* | QVLSRHGARY | PTAhKSEvYA | ELLqrIQDtA | TeFKGDFAFL | RdYaYhLGAD |
| *M. thermophila* | QVLSRHGARa | PTlkRAasYv | DLIdrIHhGA | isYgPgYEFL | RTYDYTLGAD |
| Basidio | NIIqRHGARF | PTSGaAtRiq | AaVakLQsax | xxtDPKLDFL | xnxtYxLGxD |
| Consensus | QVLSRHGARY | PTSSKSKKYS | ALI-AIQKNA | T-FKGKYAFL | KTYNYTLGAD |
| Fcp10 | QVLSRHGARY | PTSSKSKKYS | ALIEAIQKNA | TAFKGKYAFL | KTYNYTLGAD |

| | | | | | |
|---|---|---|---|---|---|
| | 101 | | | | 150 |
| *A. terreus* 9a1 | ELTPFGrNQL | rDlGaQFYeR | YNAL.TRhIn | PFVRATDAsR | VhESAEKFVE |
| *A. terreus* cbs | NLTPFGrNQL | qDlGaQFYRR | YDTL.TRhIn | PFVRAADSsR | VhESAEKFVE |
| *A. niger* var. *awamori* | DLTPFGEQEL | VNSGIKFYQR | YESL.TRnII | PFIRSSGSsR | VIASGEKFIE |
| *A. niger* NRRL3135 | DLTPFGEQEL | VNSGIKFYQR | YESL.TRnIV | PFIRSSGSsR | VIASGKKFIE |
| *A. fumigatus* 13073 | DLTPFGEQQL | VNSGIKFYQR | YKAL.ARsVV | PFIRASGSDR | VIASGEKFIE |
| *A. fumigatus* 32722 | DLTPFGEQQL | VNSGIKFYQR | YKAL.ARsVV | PFIRASGSDR | VIASGEKFIE |
| *A. fumigatus* 58128 | DLTPFGEQQL | VNSGIKFYQR | YKAL.ARsVV | PFIRASGSDR | VIASGEKFIE |
| *A. fumigatus* 26906 | DLTAFGEQQL | VNSGIKFYQR | YKAL.ARsVV | PFIRASGSDR | VIASGEKFIE |
| *A. fumigatus* 32239 | DLTPFGEQQM | VNSGIKFYQK | YKAL.AgsVV | PFIRSSGSDR | VIASGEKFIE |
| *E. nidulans* | DLTiFGENQM | VDSGaKFYRR | YKnL.ARknt | PFIRASGSDR | VVASAEKFIN |
| *T. thermophilus* | DLTPFGENQM | IQlGIKFYnH | YKSL.ARnaV | PFVRCSGSDR | VIASGrlFIE |
| *T. lanuginosa* | NLTRFGEEQM | MESGrQFYHR | YREq.AReIV | PFVRAAGSAR | VIASAEfFnr |
| *M. thermophila* | ELTRtGQQQM | VNSGIKFYRR | YRAL.ARksI | PFVRTAGqDR | VVhSAENFtQ |
| Basidio | DLvPFGAxQs | sQAGqEaFtR | YsxLvSxdnL | PFVRASGSDR | VVDSAtNWtA |
| Consensus | DLTPFGEQQM | VNSGIKFYRR | YKAL-AR-IV | PFVRASGSDR | VIASAEKFIE |

Fig. 7A

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                  Fcp10  DLTPFGEQQM VNSGIKFYRR YKAL.ARKIV PFVRASGSDR VIASAEKFIE

                         151                                                200
A. terreus 9a1           GFQTARqDDh hAnphQPSPr VDVaIPEGsA YNNTLEHSLC TAFEs...St
A. terreus cbs           GFQNARqGDP hAnphQPSPr VDVVIPEGtA YNNTLEHSIC TAFEa...St
A. niger var. awamori    GFQSTKLkDP rAqpgQSSPk IDVVISEAsS sNNTLDpGtC TvFEd...SE
A. niger NRRL3135        GFQSTKLkDP rAqpgQSSPk IDVVISEAsS sNNTLDpGtC TvFEd...SE
A. fumigatus 13073       GFQqAKLADP gAt.nRAAPa ISVIIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 32722       GFQqAKLADP gAt.nRAAPa ISVIIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 58128       GFQqAKLADP gAt.nRAAPa ISVIIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 26906       GFQqAKLADP gAt.nRAAPa ISVIIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 32239       GFQqANVADP gAt.nRAAPV ISVIIPESeT YNNTLDHSVC TnFEa...SE
E. nidulans              GFRkAQLhDh g.s.gQATPV VNVIIPEidG FNNTLDHStC vSFEn...dE
T. thermophilus          GFQSAKVlDP hSdkhDAPPt INVIIeEGpS YNNTLDtGsC PvFEd...Ss
T. lanuginosa            GFQdAKdrDP rSnkdQAePV INVIISEEtG sNNTLDgltC PAaEe...Ap
M. thermophila           GFHSAlLADR gStvrPTlPy dmVVIPETaG aNNTLHNDLC TAFEegPySt
Basidio                  GFaxA..... ..sxntxxPx LxVILSExg. .NDTLDDNMC ......PxAG

              Consensus  GFQSAKLADP -A---QASPV INVIIPEG-G YNNTLDHGLC TAFE--P-SE
                  Fcp10  GFQSAKLADP GANPHQASPV INVIIPEGAG YNNTLDHGLC TAFEE...SE

                         201                                                250
A. terreus 9a1           VGDDavANFT AVFAPAIaqR LEAdLPGVQL StDDVVNLMA MCPFETVSlT
A. terreus cbs           VGDAaADNFT AVFAPAIakR LEAdLPGVQL SADDVVNLMA MCPFETVSlT
A. niger var. awamori    LADtVEANFT AtFAPSIRqR LEndLSGVtL TDtEVtyLMD MCSFDTISts
A. niger NRRL3135        LADtVEANFT AtFvPSIRqR LEndLSGVtL TDtEVtyLMD MCSFDTISts
A. fumigatus 13073       LGDEVAANFT ALFAPdIRAR aEkhLPGVtL TDEDVVSLMD MCSFDTVArT
A. fumigatus 32722       LGDEVAANFT ALFAPdIRAR aEkhLPGVtL TDEDVVSLMD MCSFDTVArT
A. fumigatus 58128       LGDEVAANFT ALFAPdIRAR aEkhLPGVtL TDEDVVSLMD MCSFDTVArT
A. fumigatus 26906       LGDEVAANFT ALFAPdIRAR aKkhLPGVtL TDEDVVSLMD MCSFDTVArT
A. fumigatus 32239       LGDEVEANFT ALFAPAIRAR IEkhLPGVQL TDDDVVSLMD MCSFDTVArT
E. nidulans              rADEIEANFT AIMGPPIRkR LEndLPGIKL TNENVIyLMD MCSFDTMArT
T. thermophilus          gGHDaQEKFA kqFAPAIlEK IKDhLPGVDL AvsDVpyLMD LCPFETLArn
T. lanuginosa            .DptqpAEFl qVFGPRVlkK ItkhMPGVNL TlEDVplFMD LCPFDTVGsd
M. thermophila           IGDDaQDtYl StFAGPItAR VNAnLPGaNL TDADtVaLMD LCPFETVAsS
Basidio                  dSDpqxnxWl AVFAPPItAR LNAaaPGaNL TDxDaxNLxx LCPFETVS..

              Consensus  LGDDVEANFT AVFAPPIRAR LEA-LPGVNL TDEDVVNLMD MCPFDTVA-T
                  Fcp10  LGDDVEANFT AVFAPPIRAR LEAHLPGVNL TDEDVVNLMD MCPFDTVART

                         251                                                300
A. terreus 9a1           dD..Aht... ......LSPF CDLFTa..tE WtQYNYLlSL dKYYGYGGGN
A. terreus cbs           dD..Aht... ......LSPF CDLFTa..aE WtQYNYLlSL dKYYGYGGGN
A. niger var. awamori    Tv..DTK... ......LSPF CDLFTH..dE WiHYDYLQSL kKYYGHGAGN
A. niger NRRL3135        Tv..DTK... ......LSPF CDLFTH..dE WiNYDYLQSL kKYYGHGAGN
A. fumigatus 13073       SD..ASQ... ......LSPF CQLFTH..nE WkKYNYLQSL gKYYGYGAGN
A. fumigatus 32722       SD..ASQ... ......LSPF CQLFTH..nE WkKYNYLQSL gKYYGYGAGN
A. fumigatus 58128       SD..ASQ... ......LSPF CQLFTH..nE WkKYNYLQSL gKYYGYGAGN
A. fumigatus 26906       SD..ASQ... ......LSPF CQLFTH..nE WkKYNYLQSL gKYYGYGAGN
A. fumigatus 32239       AD..ASE... ......LSPF CAIFTH..nE WkKYDYLQSL gKYYGYGAGN
E. nidulans              AH..GTE... ......LSPF CAIFTE..kE WlQYDYLQSL sKYYGYGAGS
T. thermophilus          ht..DT.... ......LSPF CALsTQ..eE WqaYDYYQSL gKYYGnGGGN
T. lanuginosa            PvlfPrQ... ......LSPF CHLFTa..dD WmaYDYYyTL dKYYSHGGGS
M. thermophila           SsdpATadag ggngrpLSPF CrLFSE..sE WraYDYLQSV gKWYGYGPGN
Basidio                  .......... ...xexxSxF CDLFexxpeE FxaFxYxgdL dKFYGtGyGQ

              Consensus  SD--ATQ--- ------LSPF CDLFTH---E W-QYDYLQSL -KYYGYGAGN
                  Fcp10  SD..ATQ... ......LSPF CDLFTH..DE WIQYDYLQSL GKYYGYGAGN

                         301                                                350
A. terreus 9a1           PLGPvQGVGW aNELMARLTR A.PVHDHTCv NNTLDASPAT FPLNATLYAD
A. terreus cbs           PLGPvQGVGW aNELIARLTR S.PVHDHTCv NNTLDANPAT FPLNATLYAD
A. niger var. awamori    PLGPTQGVGY aNELIARLTH S.PVHDDTSS NHTLDSNPAT FPLNSTLYAD
A. niger NRRL3135        PLGPTQGVGY aNELIARLTH S.PVHDDTSS NHTLDSSPAT FPLNSTLYAD
```

Fig. 7B

| | | | | | |
|---|---|---|---|---|---|
| *A. fumigatus* 13073 | PLGPAQGIGF | tNELIARLTR | S.PVQDHTST | NsTLvSNPAT | FPLNATMYvD |
| *A. fumigatus* 32722 | PLGPAQGIGF | tNELIARLTR | S.PVQDHTST | NsTLvSNPAT | FPLNATMYvD |
| *A. fumigatus* 58128 | PLGPAQGIGF | tNELIARLTR | S.PVQDHTST | NsTLvSNPAT | FPLNATMYvD |
| *A. fumigatus* 26906 | PLGPAQGIGF | tNELIARLTR | S.PVQDHTST | NsTLvSNPAT | FPLNATMYvD |
| *A. fumigatus* 32239 | PLGPAQGIGF | tNELIARLTN | S.PVQDHTST | NsTLDSDPAT | FPLNATIYvD |
| *E. nidulans* | PLGPAQGIGF | tNELIARLTQ | S.PVQDNTST | NHTLDSNPAT | FPLDrkLYAD |
| *T. thermophilus* | PLGPAQGVGF | vNELIARMTH | S.PVQDYTTv | NHTLDSNPAT | FPLNATLYAD |
| *T. lanuginosa* | AFGPSRGVGF | vNELIARMTg | N1PVKDHTTv | NHTLDdNPET | FPLDAvLYAD |
| *M. thermophila* | PLGPTQGVGF | vNELLARLA. | GvPVRDgTST | NRTLDGDPrT | FPLGrPLYAD |
| Basidio | PLGPvQGVGY | iNELLARLTx | qa.VRDNTqT | NRTLDSSPxT | FPLNrTFYAD |
| Consensus | PLGPAQGVGF | -NELIARLTH | S-PVQDHTST | NHTLDSNPAT | FPLNATLYAD |
| Fcp10 | PLGPAQGVGF | VNELIARLTH | S.PVQDHTST | NHTLDSNPAT | FPLNATLYAD |

| | 351 | | | | 400 |
|---|---|---|---|---|---|
| *A. terreus* 9a1 | FSHDSnLVSI | FWALGLYNGT | aPLSqTSVE. | .SvsQTDGYA | AAWTVPFAAR |
| *A. terreus* cbs | FSHDSnLVSI | FWALGLYNGT | kPLSqTTVE. | .ditrTDGYA | AAWTVPFAAR |
| *A. niger* var. *awamori* | FSHDNGIISI | LFALGLYNGT | kPLSTTTVE. | .NitQTDGFS | SAWTVPFASR |
| *A. niger* NRRL3135 | FSHDNGIISI | LFALGLYNGT | kPLSTTTVE. | .NitQTDGFS | SAWTVPFASR |
| *A. fumigatus* 13073 | FSHDNSMVSI | FFALGLYNGT | ePLSrTSVE. | .SaKElDGYS | ASWvVPFGAR |
| *A. fumigatus* 32722 | FSHDNSMVSI | FFALGLYNGT | gPLSrTSVE. | .SaKElDGYS | ASWvVPFGAR |
| *A. fumigatus* 58128 | FSHDNSMVSI | FFALGLYNGT | ePLSrTSVE. | .SaKElDGYS | ASWvVPFGAR |
| *A. fumigatus* 26906 | FSHDNSMVSI | FFALGLYNGT | ePLSrTSVE. | .SaKElDGYS | ASWvVPFGAR |
| *A. fumigatus* 32239 | FSHDNGMIPI | FFAMGLYNGT | ePLSqTSeE. | .StKESNGYS | ASWAVPFGAR |
| *E. nidulans* | FSHDNSMISI | FFAMGLYNGT | qPLSmdSVE. | .SiQEmDGYA | ASWTVPFGAR |
| *T. thermophilus* | FSHDNTMtSI | FaALGLYNGT | akLSTTeIK. | .SiEETDGYS | AAWTVPFGGR |
| *T. lanuginosa* | FSHDNTMtGI | FsAMGLYNGT | kPLSTSkIQP | pTgAAADGYA | ASWTVPFAAR |
| *M. thermophila* | FSHDNdMMGV | LgALGaYDGv | pPLdkTA..R | rdpEElGGYA | ASWAVPFAAR |
| Basidio | FSHDNqMVAI | FsAMGLFNqS | aPLdPSxpDP | nrt.....Wv | TSklVPFSAR |
| Consensus | FSHDNTMVSI | FFALGLYNGT | -PLSTTSVEP | -S-EETDGYA | ASWTVPFAAR |
| Fcp10 | FSHDNTMVSI | FFALGLYNGT | KPLSTTSVE. | .SIEETDGYA | ASWTVPFAAR |

| | 401 | | | | 450 |
|---|---|---|---|---|---|
| *A. terreus* 9a1 | AYVEMMQC.. | ra........ | .....EKEPL | VRVLVNDRVM | PLHGCPtDKL |
| *A. terreus* cbs | AYIEMMQC.. | ra........ | .....EKQPL | VRVLVNDRVM | PLHGCAVDNL |
| *A. niger* var. *awamori* | lYVEMMQC.. | Qa........ | .....EQEPL | VRVLVNDRVV | PLHGCPIDaL |
| *A. niger* NRRL3135 | lYVEMMQC.. | Qa........ | .....EQEPL | VRVLVNDRVV | PLHGCPVDaL |
| *A. fumigatus* 13073 | AYfEtMQC.. | Ks........ | .....EKEPL | VRaLINDRVV | PLHGCDVDKL |
| *A. fumigatus* 32722 | AYfEtMQC.. | Ks........ | .....EKEPL | VRaLINDRVV | PLHGCDVDKL |
| *A. fumigatus* 58128 | AYfEtMQC.. | Ks........ | .....EKESL | VRaLINDRVV | PLHGCDVDKL |
| *A. fumigatus* 26906 | AYfEtMQC.. | Ks........ | .....EKEPL | VRaLINDRVV | PLHGCDVDKL |
| *A. fumigatus* 32239 | AYfEtMQC.. | Ks........ | .....EKEPL | VRaLINDRVV | PLHGCAVDKL |
| *E. nidulans* | AYfELMQC.. | E......... | .....KKEPL | VRVLVNDRVV | PLHGCAVDKF |
| *T. thermophilus* | AYIEMMQC.. | Dd........ | .....sDEPV | VRVLVNDRVV | PLHGCEVDsL |
| *T. lanuginosa* | AYVELLRC.. | Etetsseeee | EG...EDEPF | VRVLVNDRVV | PLHGCrVDRW |
| *M. thermophila* | iYVEkMRC.. | sggggggggg | EGrqeKDEeM | VRVLVNDRVM | TLkGCGaDEr |
| Basidio | mvVErLxCxx | xgtxxxxxxx | xxxxxxxxxx | VRVLVNDaVq | PLEfCGgDxd |
| Consensus | AYVEMMQC-- | E--------- | EG---EKEPL | VRVLVNDRVV | PLHGCGVDKL |
| Fcp10 | AYVEMMQC.. | EA........ | .....EKEPL | VRVLVNDRVV | PLHGCGVDKL |

Fig. 7C

| | 451 | | 482 |
|---|---|---|---|
| *A. terreus* 9a1 | GRCKrDAFVA GLSFAQAG.. | GNWADCF--- | -- |
| *A. terreus* cbs | GRCKrDDFVE GLSFARAG.. | GNWAECF--- | -- |
| *A. niger* var. *awamori* | GRCtrDsFVr GLSFARSG.. | GDWAECsA-- | -- |
| *A. niger* NRRL3135 | GRCtrDsFVr GLSFARSG.. | GDWAECFA-- | -- |
| *A. fumigatus* 13073 | GRCKlNDFVK GLSWARSG.. | GNWGECFS-- | -- |
| *A. fumigatus* 32722 | GRCKlNDFVK GLSWARSG.. | GNWGECFS-- | -- |
| *A. fumigatus* 58128 | GRCKlNDFVK GLSWARSG.. | GNWGECFS-- | -- |
| *A. fumigatus* 26906 | GRCKlNDFVK GLSWARSG.. | GNWGECFS-- | -- |
| *A. fumigatus* 32239 | GRCKlKDFVK GLSWARSG.. | GNSEQSFS-- | -- |
| *E. nidulans* | GRCtlDDWVE GLNFARSG.. | GNWKtCFTl- | -- |
| *T. thermophilus* | GRCKrDDFVr GLSFARqG.. | GNWEGCYAas | e- |
| *T. lanuginosa* | GRCRrDEWIK GLTFARqG.. | GHWDrCF--- | -- |
| *M. thermophila* | GmCtlErFIE SMAFARGN.. | GKWDlCFA-- | -- |
| Basidio | GxCtlDAFVE SqxYAReDgq | GDFEKCFAtp | xx |
| Consensus | GRCK-DDFVE GLSFARSG-- | GNWEECFA-- | -- |
| Fcp10 | GRCKRDDFVE GLSFARSG.. | GNWEECFA.. | .. |

Fig. 7D

| | 1 | | | | 50 |
|---|---|---|---|---|---|
| *P. involutus (phyA1)* | ---------- | -FPipeseqR | nWSPYSPYFP | LAEykA.... | pPaGCQInqV |
| *P. involutus (phyA2)* | ---------- | -FsipeseqR | nWSPYSPYFP | LAEykA.... | pPaGCeInqV |
| *T. pubescens* | ---------- | -LDvtRDVqQ | sWSmYSPYFP | aAtyvA.... | pPaSCQInqV |
| *A. pediades* | ---------- | -pffpPQIqD | sWAaYTPYYP | VqAyTP.... | pPKDCKITqV |
| *P. lycii* | ---------- | -LPipAQnTs | nWGPYdPFFP | VEpyAA.... | pPEGCtVTqV |
| *A. terreus* 9a1 | KhsdCNSVDh | GYQCfPELSH | kWGlYAPYFS | LqDESPFPlD | VPEDCHITFV |
| *A. terreus* cbs | NhsdCtSVDr | GYQCfPELSH | kWGlYAPYFS | LqDESPFPlD | VPDDCHITFV |
| *A. niger* var. *awamori* | NqsTCDTVDq | GYQCfSEtSH | LWGQYAPFFS | LANESAISPD | VPaGCRVTFa |
| *A. niger* T213 | NqsSCDTVDq | GYQCfSEtSH | LWGQYAPFFS | LANESvISPD | VPaGCRVTFa |
| *A. niger* NRRL3135 | NqsSCDTVDq | GYQCfSEtSH | LWGQYAPFFS | LANESvISPE | VPaGCRVTFa |
| *A. fumigatus* ATCC13073 | GSkSCDTVDl | GYQCsPAtSH | LWGQYSPFFS | LEDElSVSSK | LPKDCRITLV |
| *A. fumigatus* ATCC32722 | GSkSCDTVDl | GYQCsPAtSH | LWGQYSPFFS | LEDElSVSSK | LPKDCRITLV |
| *A. fumigatus* ATCC58128 | GSkSCDTVDl | GYQCsPAtSH | LWGQYSPFFS | LEDElSVSSK | LPKDCRITLV |
| *A. fumigatus* ATCC26906 | GSkSCDTVDl | GYQCsPAtSH | LWGQYSPFFS | LEDElSVSSK | LPKDCRITLV |
| *A. fumigatus* ATCC32239 | GSkACDTVEl | GYQCsPGtSH | LWGQYSPFFS | LEDElSVSSD | LPKDCRVTFV |
| *E. nidulans* | QNHSCNTaDg | GYQCfPNVSH | VWGQYSPYFS | IEQESAISeD | VPhGCeVTFV |
| *T. thermophilus* | DSHSCNTVEg | GYQCrPEISH | sWGQYSPFFS | LADQSEISPD | VPQNCKITFV |
| *T. lanuginosa* | ---------- | ----nvDIAR | hWGQYSPFFS | LAEvSEISPA | VPKGCRVeFV |
| *M. thermophila* | ESRPCDTpDl | GFQCgTAISH | FWGQYSPYFS | VPsElDaS.. | IPDDCeVTFa |
| Consensus Seq. 11 | NSHSCDTVD- | GYQC-PEISH | LWGQYSPFFS | LADESAISPD | VPKGCRVTFV |

| | 51 | | | | 100 |
|---|---|---|---|---|---|
| *P. involutus (phyA1)* | NIIqRHGARF | PTSGaTtRik | AgLtKLQgvq | nftDAKFnFI | KSFKYdLGns |
| *P. involutus (phyA2)* | NIIqRHGARF | PTSGaAtRik | AgLsKLQsvq | nftDPKFDFI | KSFtYdLGTs |
| *T. pubescens* | HIIqRHGARF | PTSGaAKRiq | TaVAKLKaaS | nytDPlLAFV | tnYtYSLGqD |
| *A. pediades* | NIIqRHGARF | PTSGaGtRiq | AaVKKLQsak | TytDPRLDFL | tnYtYTLGhD |
| *P. lycii* | NLIqRHGARW | PTSGarsRqv | AaVAKIQmar | PftDPKYEFL | NdFvYkFGvA |
| *A. terreus* 9a1 | QVLARHGARs | PThSKTKaYA | AtIAaIQKSA | TaFpGKYAFL | QSYNYSLDSE |
| *A. terreus* cbs | QVLARHGARs | PTdSKTKaYA | AtIAaIQKNA | TaLpGKYAFL | KSYNYSMGSE |
| *A. niger* var. *awamori* | QVLSRHGARY | PTeSKGKKYS | ALIEeIQQNv | TtFDGKYAFL | KTYNYSLGAD |
| *A. niger* T213 | QVLSRHGARY | PTeSKGKKYS | ALIEeIQQNv | TtFDGKYAFL | KTYNYSLGAD |
| *A. niger* NRRL3135 | QVLSRHGARY | PTdSKGKKYS | ALIEeIQQNA | TtFDGKYAFL | KTYNYSLGAD |
| *A. fumigatus* ATCC13073 | QVLSRHGARY | PTSSKSKKYk | kLVtaIQaNA | TdFKGKFAFL | KTYNYTLGAD |
| *A. fumigatus* ATCC32722 | QVLSRHGARY | PTSSKSKKYk | kLVtaIQaNA | TdFKGKFAFL | KTYNYTLGAD |
| *A. fumigatus* ATCC58128 | QVLSRHGARY | PTSSKSKKYk | kLVtaIQaNA | TdFKGKFAFL | KTYNYTLGAD |
| *A. fumigatus* ATCC26906 | QVLSRHGARY | PTSSKSKKYk | kLVtaIQaNA | TdFKGKFAFL | KTYNYTLGAD |
| *A. fumigatus* ATCC32239 | QVLSRHGARY | PTASKSKKYk | kLVtaIQKNA | TeFKGKFAFL | ETYNYTLGAD |
| *E. nidulans* | QVLSRHGARY | PTeSKSKaYS | GLIEaIQKNA | TsFwGQYAFL | ESYNYTLGAD |
| *T. thermophilus* | QLLSRHGARY | PTSSKTElYS | qLIsRIQKtA | TaYKGyYAFL | KdYrYqLGAN |
| *T. lanuginosa* | QVLSRHGARY | PTAhKSEvYA | ELLQRIQDtA | TeFKGDFAFL | RdYaYhLGAD |
| *M. thermophila* | QVLSRHGARa | PTlkRAasYv | DLIDRIHhGA | isYgPgYEFL | RTYDYTLGAD |
| Consensus Seq. 11 | QVLSRHGARY | PTSSKSKKYS | ALIERIQKNA | T-FKGKYAFL | KTYNYTLGAD |

101

150

| | | | | | |
|---|---|---|---|---|---|
| *P. involutus (phyA1)* | DLvPFGAaQs | fDAGqEaFaR | YskLvSKNnL | PFIRAdGSDR | VVDSAtNWtA |
| *P. involutus (phyA2)* | DLvPFGAaQs | fDAGLEvFaR | YskLvSsDnL | PFIRSdGSDR | VVDTAtNWtA |
| *T. pubescens* | sLveLGAtQs | sEAGqEaFtR | YsSLvSaDeL | PFVRASGSDR | VVATANNWtA |
| *A. pediades* | DLvPFGAlQs | sQAGeEtFQR | YsfLvSKEnL | PFVRASSSNR | VVDSAtNWtE |
| *P. lycii* | DLlPFGANQs | hQTGtDMYtR | YsTLfEgGdV | PFVRAAGdQR | VVDSStNWtA |
| *A. terreus* 9a1 | ELTPFGrNQL | rDlGaQFYeR | YNAL.TRHIn | PFVRATDAsR | VhESAEKFVE |
| *A. terreus* cbs | NLTPFGrNQL | qDlGaQFYRR | YDTL.TRHIn | PFVRAADSsR | VhESAEKFVE |
| *A. niger* var. *awamori* | DLTPFGEQEL | VNSGIKFYQR | YESL.TRNII | PFIRSSGSsR | VIASGEKFIE |
| *A. niger* T213 | DLTPFGEQEL | VNSGIKFYQR | YESL.TRNII | PFIRSSGSsR | VIASGEKFIE |
| *A. niger* NRRL3135 | DLT?FGEQEL | VNSGIKFYQR | YESL.TRNIV | PFIRSSGSsR | VIASGKKFIE |
| *A. fumigatus* ATCC13073 | DLTPFGEQQL | VNSGIKFYQR | YKAL.ARSVV | PFIRASGSDR | VIASGEKFIE |
| *A. fumigatus* ATCC32722 | DLTPFGEQQL | VNSGIKFYQR | YKAL.ARSVV | PFIRASGSDR | VIASGEKFIE |
| *A. fumigatus* ATCC58128 | DLTPFGEQQL | VNSGIKFYQR | YKAL.ARSVV | PFIRASGSDR | VIASGEKFIE |

Fig. 8A

| | | | | | |
|---|---|---|---|---|---|
| A. *fumigatus* ATCC26906 | DLTAFGEQQL | VNSGIKFYQR | YKAL.ARSVV | PFIRASGSDR | VIASGEKFIE |
| A. *fumigatus* ATCC32239 | DLTPFGEQQM | VNSGIKFYQK | YKAL.AgSVV | PFIRSSGSDR | VIASGEKFIE |
| E. *nidulans* | DLTiFGENQM | VDSGaKFYRR | YKnL.ARKnt | PFIRASGSDR | VVASAEKFIN |
| T. *thermophilus* | DLTPFGENQM | IQlGIKFYnH | YKSL.ARNaV | PFVRCSGSDR | VIASGrlFIE |
| T. *lanuginosa* | NLTRFGEEQM | MESGrQFYHR | YREq.AREIV | PFVRAAGSAR | VIASAEfFnr |
| M. *thermophila* | ELTRtGQQQM | VNSGIKFYRR | YRAL.ARKsI | PFVRTAGqDR | VVhSAENFtQ |
| **Consensus Seq. 11** | **DLTPFGENQM** | **VNSGIKFYRR** | **YKAL-ARNIV** | **PFVRASGSDR** | **VIASAEKFIE** |

| | 151 | | | | 200 |
|---|---|---|---|---|---|
| P. *involutus* (*phyA1*) | GFaSA..... | ..shNtvqPk | LNLILPQ..T | gNDTLEDNMC | PAaGD..... |
| P. *involutus* (*phyA2*) | GFaSA..... | ..srNaiqPk | LDLILPQ..T | gNDTLEDNMC | PAaGE..... |
| T. *pubescens* | GFalA..... | ..ssNsiTPV | LSVIISE..A | gNDTLDDNMC | PAaGD..... |
| A. *pediades* | GFsAA..... | ..shHvlNPI | LfVILSE..S | LNDTLDDAMC | PnaGs..... |
| P. *lycii* | GFgdA..... | ..sgEtvlPt | LQVVLQE..E | gNcTLcNNMC | PnevD..... |
| A. *terreus* 9a1 | GFQTARqDDh | hAnpHQPSPr | VDVaIPEGSA | YNNTLEHSLC | TAFEs...ST |
| A. *terreus* cbs | GFQNARqGDP | hAnpHQPSPr | VDVVIPEGTA | YNNTLEHSIC | TAFEA...ST |
| A. *niger* var. *awamori* | GFQSTKLkDP | rAqpgQSSPk | IDVVISEASS | sNNTLDpGtC | TvFED...Se |
| A. *niger* T213 | GFQSTKLkDP | rAqpgQSSPk | IDVVISEASS | sNNTLDpGtC | TvFED...Se |
| A. *niger* NRRL3135 | GFQSTKLkDP | rAqpgQSSPk | IDVVISEASS | sNNTLDpGtC | TvFED...Se |
| A. *fumigatus* ATCC13073 | GFQqAKLADP | gAt.NRAAPa | ISVIIPESeT | FNNTLDHGVC | TkFEA...Sq |
| A. *fumigatus* ATCC32722 | GFQqAKLADP | gAt.NRAAPa | ISVIIPESeT | FNNTLDHGVC | TkFEA...Sq |
| A. *fumigatus* ATCC58128 | GFQqAKLADP | gAt.NRAAPa | ISVIIPESeT | FNNTLDHGVC | TkFEA...Sq |
| A. *fumigatus* ATCC26906 | GFQqAKLADP | gAt.NRAAPa | ISVIIPESeT | FNNTLDHGVC | TkFEA...Sq |
| A. *fumigatus* ATCC32239 | GFQqANVADP | gAt.NRAAPV | ISVIIPESeT | YNNTLDHSVC | TnFEA...Se |
| E. *nidulans* | GFRkAQLhDh | g.s.gQATPV | VNVIIPEidG | FNNTLDHStC | vSFEN...de |
| T. *thermophilus* | GFQSAKVlDP | hSdkHDAPPt | INVIIeEGPS | YNNTLDtGsC | PvFED...SS |
| T. *lanuginosa* | GFQdAKdrDP | rSnkDQAePV | INVIISEETG | sNNTLDgltC | PAaEE...AP |
| M. *thermophila* | GFHSAlLADR | gStvRPTlPy | dmVVIPETAG | aNNTLHNDLC | TAFEEgpyST |
| **Consensus Seq. 11** | **GFQSAKLADP** | **-A--HQASPV** | **INVIIPEGSG** | **YNNTLDHGLC** | **TAFED---ST** |

| | 201 | | | | 250 |
|---|---|---|---|---|---|
| P. *involutus* (*phyA1*) | .SDpqvnaWl | AVafPSItAR | LNAaaPSVNL | TDtDafNLVs | LCAFlTVSK. |
| P. *involutus* (*phyA2*) | .SDpqvDaWl | AsafPSVtAQ | LNAaaPGaNL | TDADafNLVs | LCPFmTVSK. |
| T. *pubescens* | .SDpqvnQWl | AqFAPPMtAR | LNAgaPGaNL | TDtDtyNLLt | LCPFETVAt. |
| A. *pediades* | .SDpqtGiWT | SIYGTPIanR | LNqqaPGaNI | TAADVsNLIp | LCAFETIvK. |
| P. *lycii* | .GDESt.tWl | GVFAPnItAR | LNAaaPSaNL | SDsDaLtLMD | MCPFDTLSs. |
| A. *terreus* 9a1 | VGDDAvANFT | AVFAPAIaqR | LEAdLPGVQL | StDDVVNLMA | MCPFETVSlT |
| A. *terreus* cbs | VGDAAADNFT | AVFAPAIakR | LEAdLPGVQL | SADDVVNLMA | MCPFETVSlT |
| A. *niger* var. *awamori* | LADtvEANFT | AtFAPSIRqR | LEndLSGVtL | TDtEVtyLMD | MCSFDTIStS |
| A. *niger* T213 | LADtvEANFT | AtFAPSIRqR | LEndLSGVtL | TDtEVtyLMD | MCSFDTIStS |
| A. *niger* NRRL3135 | LADtvEANFT | AtFvPSIRqR | LEndLSGVtL | TDtEVtyLMD | MCSFDTIStS |
| A. *fumigatus* ATCC13073 | LGDEvAANFT | ALFAPdIRAR | aEkhLPGVtL | TDEDVVSLMD | MCSFDTVART |
| A. *fumigatus* ATCC32722 | LGDEvAANFT | ALFAPdIRAR | aEkhLPGVtL | TDEDVVSLMD | MCSFDTVART |
| A. *fumigatus* ATCC58128 | LGDEvAANFT | ALFAPdIRAR | aKkhLPGVtL | TDEDVVSLMD | MCSFDTVART |
| A. *fumigatus* ATCC26906 | LGDEvAANFT | ALFAPdIRAR | IEkhLPGVQL | TDDDVVSLMD | MCSFDTVART |
| A. *fumigatus* ATCC32239 | LGDEvEANFT | ALFAPAIRAR | LEndLPGIKL | TNENVIyLMD | MCSFDTMART |
| E. *nidulans* | rADEiEANFT | AIMGPPIRkR | IKDhLPGVDL | AvsDVpyLMD | LCPFETLARn |
| T. *thermophilus* | gGHDAQEKFA | kqFAPAIlEK | ItkhMPGVNL | TlEDVplFMD | LCPFDTVGsd |
| T. *lanuginosa* | .DptqpAEFl | qVFGPRVlkK | ItkhMPGVNL | TlEDVplFMD | LCPFDTVGsd |
| M. *thermophila* | IGDDAQDtYl | StFAGPItAR | VNAnLPGaNL | TDADtVaLMD | LCPFETVAsS |
| **Consensus Seq. 11** | **LGDDAEANFT** | **AVFAPPIRAR** | **LEA-LPGVNL** | **TDEDVVNLMD** | **MCPFDTVART** |

| | 251 | | | | 300 |
|---|---|---|---|---|---|
| P. *involutus* (*phyA1*) | .......... | ....ekkSdF | CtLFegiPGs | FeaFAYggdL | dKFYGtGyGQ |
| P. *involutus* (*phyA2*) | .......... | ....eqkSdF | CtLFegiPGs | FeaFAYagdL | dKFYGtGyGQ |
| T. *pubescens* | .......... | ....errSeF | CDIYeelqAE | .daFAYnadL | dKFYGtGyGQ |
| A. *pediades* | .......... | ....etpSPF | CNLF..TPEE | FaQFEYFgdL | dKFYGtGyGQ |
| P. *lycii* | .......... | ....gnaSPF | CDLF..TAEE | YvsYEYYydL | dKYYGtGPGN |
| A. *terreus* 9a1 | dD..Aht... | ......LSPF | CDLF..TAtE | WtQYNYLlSL | dKYYGYGGGN |
| A. *terreus* cbs | dD..Aht... | ......LSPF | CDLF..TAAE | WtQYNYLlSL | dKYYGYGGGN |
| A. *niger* var. *awamori* | Tv..DTK... | ......LSPF | CDLF..ThDE | WiHYDYLQSL | kKYYGHGAGN |

Fig. 8B

| | | | | | | |
|---|---|---|---|---|---|---|
| *A. niger* T213 | Tv..DTK... | ......LSPF | CDLF..ThDE | WiHYDYLRSL | kKYYGHGAGN | |
| *A. niger* NRRL3135 | Tv..DTK... | ......LSPF | CDLF..ThDE | WiNYDYLQSL | kKYYGHGAGN | |
| *A. fumigatus* ATCC13073 | SD..ASQ... | ......LSPF | CQLF..ThNE | WkKYNYLQSL | gKYYGYGAGN | |
| *A. fumigatus* ATCC32722 | SD..ASQ... | ......LSPF | CQLF..ThNE | WkKYNYLQSL | gKYYGYGAGN | |
| *A. fumigatus* ATCC58128 | SD..ASQ... | ......LSPF | CQLF..ThNE | WkKYNYLQSL | gKYYGYGAGN | |
| *A. fumigatus* ATCC26906 | SD..ASQ... | ......LSPF | CQLF..ThNE | WkKYNYLQSL | gKYYGYGAGN | |
| *A. fumigatus* ATCC32239 | AD..ASE... | ......LSPF | CAIF..ThNE | WkKYDYLQSL | gKYYGYGAGN | |
| *E. nidulans* | AH..GTE... | ......LSPF | CAIF..TEKE | WlQYDYLQSL | sKYYGYGAGS | |
| *T. thermophilus* | ht..DT.... | ......LSPF | CALs..TqEE | WqaYDYYQSL | gKYYGnGGGN | |
| *T. lanuginosa* | PvlfPrQ... | ......LSPF | CHLF..TADD | WmaYDYYyTL | dKYYSHGGGS | |
| *M. thermophila* | SsdpATadag | ggngrpLSPF | CrLF..SEsE | WraYDYLQSV | gKWYGYGPGN | |
| Consensus Seq. 11 | SD--ATQ--- | ------LSPF | CDLF--TADE | W-QYDYLQSL | -KYYGYGAGN | |

| | 301 | | | | 350 |
|---|---|---|---|---|---|
| *P. involutus* (*phyA1*) | eLGPvQGVGY | vNELIARLTN | S.AVRDNTqT | NRTLDASPvT | FPLNkTFYAD |
| *P. involutus* (*phyA2*) | ALGPvQGVGY | iNELLARLTN | S.AVNDNTqT | NRTLDAaPDT | FPLNkTMYAD |
| *T. pubescens* | PLGPvQGVGY | iNELIARLTa | q.nVsDHTqT | NsTLDSSPET | FPLNrTLYAD |
| *A. pediades* | PLGPvQGVGY | iNELLARLTE | m.PVRDNTqT | NRTLDSSPlT | FPLDrSIYAD |
| *P. lycii* | ALGPvQGVGY | vNELLARLTg | q.AVRDETqT | NRTLDSDPAT | FPLNrTFYAD |
| *A. terreus* 9a1 | PLGPvQGVGW | aNELMARLTR | A.PVHDHTCv | NNTLDASPAT | FPLNATLYAD |
| *A. terreus* cbs | PLGPvQGVGW | aNELIARLTR | S.PVHDHTCv | NNTLDANPAT | FPLNATLYAD |
| *A. niger* var. *awamori* | PLGPTQGVGY | aNELIARLTH | S.PVHDDTSS | NHTLDSNPAT | FPLNSTLYAD |
| *A. niger* T213 | PLGPTQGVGY | aNELIARLTH | S.PVHDDTSS | NHTLDSNPAT | FPLNSTLYAD |
| *A. niger* NRRL3135 | PLGPTQGVGY | aNELIARLTH | S.PVHDDTSS | NHTLDSSPAT | FPLNSTLYAD |
| *A. fumigatus* ATCC13073 | PLGPAQGIGF | tNELIARLTR | S.PVQDHTST | NsTLvSNPAT | FPLNATMYvD |
| *A. fumigatus* ATCC32722 | PLGPAQGIGF | tNELIARLTR | S.PVQDHTST | NsTLvSNPAT | FPLNATMYvD |
| *A. fumigatus* ATCC58128 | PLGPAQGIGF | tNELIARLTR | S.PVQDHTST | NsTLvSNPAT | FPLNATMYvD |
| *A. fumigatus* ATCC26906 | PLGPAQGIGF | tNELIARLTR | S.PVQDHTST | NsTLvSNPAT | FPLNATMYvD |
| *A. fumigatus* ATCC32239 | PLGPAQGIGF | tNELIARLTN | S.PVQDHTST | NsTLDSDPAT | FPLNATIYvD |
| *E. nidulans* | PLGPAQGIGF | tNELIARLTQ | S.PVQDNTST | NHTLDSNPAT | FPLDrkLYAD |
| *T. thermophilus* | PLGPAQGVGF | vNELIARMTH | S.PVQDYTTv | NHTLDSNPAT | FPLNATLYAD |
| *T. lanuginosa* | AFGPSRGVGF | vNELIARMTg | NlPVKDHTTv | NHTLDdNPET | FPLDAvLYAD |
| *M. thermophila* | PLGPTQGVGF | vNELLARLA. | GvPVRDgTST | NRTLDGDPrT | FPLGrPLYAD |
| Consensus Seq. 11 | PLGPAQGVGF | -NELIARLTH | S-PVQDHTST | NHTLDSNPAT | FPLNATLYAD |

| | 351 | | | | 400 |
|---|---|---|---|---|---|
| *P. involutus* (*phyA1*) | FSHDNlMVAV | FsAMGLFrqP | aPLSTSvpNP | wrt.....Wr | TSSlVPFSGR |
| *P. involutus* (*phyA2*) | FSHDNlMVAV | FsAMGLFrqS | aPLSTSTpDP | nrt.....Wl | TSSvVPFSAR |
| *T. pubescens* | FSHDNqMVAI | FsAMGLFNqS | aPLdPTTpDP | art.....Fl | vkkiVPFSAR |
| *A. pediades* | LSHDNqMIAI | FsAMGLFNqS | sPLdPSfpNP | krt.....Wv | TSRltPFSAR |
| *P. lycii* | FSHDNTMVPI | FaALGLFNAT | a.LdPlkpDe | nrl.....Wv | DSklVPFSGH |
| *A. terreus* 9a1 | FSHDSnLVSI | FWALGLYNGT | aPLSqTSVES | Vs..QTDGYA | AAWTVPFAAR |
| *A. terreus* cbs | FSHDSnLVSI | FWALGLYNGT | KPLSqTTVEd | It..rTDGYA | AAWTVPFAAR |
| *A. niger* var. *awamori* | FSHDNGIISI | LFALGLYNGT | KPLSTTTVEN | It..QTDGFS | SAWTVPFASR |
| *A. niger* T213 | FSHDNGIISI | LFALGLYNGT | KPLSTTTVEN | It..QTDGFS | SAWTVPFASR |
| *A. niger* NRRL3135 | FSHDNGIISI | LFALGLYNGT | KPLSTTTVEN | It..QTDGFS | SAWTVPFASR |
| *A. fumigatus* ATCC13073 | FSHDNSMVSI | FFALGLYNGT | EPLSrTSVES | ak..ElDGYS | ASWvVPFGAR |
| *A. fumigatus* ATCC32722 | FSHDNSMVSI | FFALGLYNGT | gPLSrTSVES | ak..ElDGYS | ASWvVPFGAR |
| *A. fumigatus* ATCC58128 | FSHDNSMVSI | FFALGLYNGT | EPLSrTSVES | ak..ElDGYS | ASWvVPFGAR |
| *A. fumigatus* ATCC26906 | FSHDNSMVSI | FFALGLYNGT | EPLSrTSVES | ak..ElDGYS | ASWvVPFGAR |
| *A. fumigatus* ATCC32239 | FSHDNGMIPI | FFAMGLYNGT | EPLSqTSeES | tk..ESNGYS | ASWAVPFGAR |
| *E. nidulans* | FSHDNSMISI | FFAMGLYNGT | QPLSmdSVES | Iq..EmDGYA | ASWTVPFGAR |
| *T. thermophilus* | FSHDNTMtSI | FaALGLYNGT | akLSTTeIKS | Ie..ETDGYS | AAWTVPFGGR |
| *T. lanuginosa* | FSHDNTMtGI | FsAMGLYNGT | KPLSTSkIQP | ptgaAADGYA | ASWTVPFAAR |
| *M. thermophila* | FSHDNdMMGV | LgALGaYDGv | pPLdkTArrd | ..peElGGYA | ASWAVPFAAR |
| Consensus Seq. 11 | FSHDNTMVSI | FFALGLYNGT | KPLSTTSVES | I---ETDGYA | ASWTVPFAAR |

| | 401 | | | | 450 |
|---|---|---|---|---|---|
| *P. involutus* (*phyA1*) | mvVErLsC.. | fGt....... | ........Tk | VRVLVQDQVq | PLEfCGgDRn |
| *P. involutus* (*phyA2*) | maVErLsC.. | AGt....... | ........Tk | VRVLVQDQVq | PLEfCGgDQd |
| *T. pubescens* | mvVErLDC.. | GGa....... | ........Qs | VRLLVNDaVq | PLafCGaDts |

Fig. 8C

| | | | | | |
|---|---|---|---|---|---|
| *A. pediades* | mvtErLlCQr | DGtGsGGpsr | imrNgnvQTF | VRILVNDaLq | PLkfCGgDmd |
| *P. lycii* | mtVEkLaC.. | .......... | .....sgKea | VRVLVNDaVq | PLEfCGg.vd |
| *A. terreus* 9a1 | AYVEMMQCrA | .......... | ..EK...EPL | VRVLVNDRVM | PLHGCPtDKL |
| *A. terreus* cbs | AYIEMMQCrA | .......... | ..EK...QPL | VRVLVNDRVM | PLHGCAVDNL |
| *A. niger* var. *awamori* | lYVEMMQCQA | .......... | ..EQ...EPL | VRVLVNDRVV | PLHGCPIDaL |
| *A. niger* T213 | lYVEMMQCQA | .......... | ..EQ...EPL | VRVLVNDRVV | PLHGCPIDaL |
| *A. niger* NRRL3135 | lYVEMMQCQA | .......... | ..EQ...EPL | VRVLVNDRVV | PLHGCPVDaL |
| *A. fumigatus* ATCC13073 | AYfEtMQCKS | .......... | ..EK...EPL | VRaLINDRVV | PLHGCDVDKL |
| *A. fumigatus* ATCC32722 | AYfEtMQCKS | .......... | ..EK...EPL | VRaLINDRVV | PLHGCDVDKL |
| *A. fumigatus* ATCC58128 | AYfEtMQCKS | .......... | ..EK...ESL | VRaLINDRVV | PLHGCDVDKL |
| *A. fumigatus* ATCC26906 | AYfEtMQCKS | .......... | ..EK...EPL | VRaLINDRVV | PLHGCDVDKL |
| *A. fumigatus* ATCC32239 | AYfEtMQCKS | .......... | ..EK...EPL | VRaLINDRVV | PLHGCAVDKL |
| *E. nidulans* | AYfELMQCE. | .......... | ..KK...EPL | VRVLVNDRVV | PLHGCAVDKF |
| *T. thermophilus* | AYIEMMQCDD | .......... | ..sD...EPV | VRVLVNDRVV | PLHGCEVDsL |
| *T. lanuginosa* | AYVELLRCET | ETsSeEEeEG | ..ED...EPF | VRVLVNDRVV | PLHGCrVDRW |
| *M. thermophila* | iYVEkMRCsG | GGgGgGGgEG | ..rQekdEeM | VRVLVNDRVM | TLkGCGaDEr |
| Consensus Seq. 11 | AYVEMMQCEA | GG-G-GG-EG | --EK---EPL | VRVLVNDRVV | PLHGCGVDKL |

| | 451 | | | 482 |
|---|---|---|---|---|
| *P. involutus* (*phyA1*) | GlCtLAKFVE | SqTFARSDga | GDFEKCFAts | a~ |
| *P. involutus* (*phyA2*) | GlCaLDKFVE | SqAYARSGga | GDFEKCLAtt | v~ |
| *T. pubescens* | GvCtLDAFVE | SqAYARNDge | GDFEKCFAt~ | ~~ |
| *A. pediades* | SlCtLEAFVE | SqkYAReDgq | GDFEKCFD~~ | ~~ |
| *P. lycii* | GvCELsAFVE | SqTYAReNgq | GDFAKCgfvp | se |
| *A. terreus* 9a1 | GRCKrDAFVA | GLSFAQAG.. | GNWADCF~~~ | ~~ |
| *A. terreus* cbs | GRCKrDDFVE | GLSFARAG.. | GNWAECF~~~ | ~~ |
| *A. niger* var. *awamori* | GRCtrDsFVr | GLSFARSG.. | GDWAECsA~~ | ~~ |
| *A. niger* T213 | GRCtrDsFVr | GLSFARSG.. | GDWAECFA~~ | ~~ |
| *A. niger* NRRL3135 | GRCtrDsFVr | GLSFARSG.. | GDWAECFA~~ | ~~ |
| *A. fumigatus* ATCC13073 | GRCKLNDFVK | GLSWARSG.. | GNWGECFS~~ | ~~ |
| *A. fumigatus* ATCC32722 | GRCKLNDFVK | GLSWARSG.. | GNWGECFS~~ | ~~ |
| *A. fumigatus* ATCC58128 | GRCKLNDFVK | GLSWARSG.. | GNWGECFS~~ | ~~ |
| *A. fumigatus* ATCC26906 | GRCKLNDFVK | GLSWARSG.. | GNWGECFS~~ | ~~ |
| *A. fumigatus* ATCC32239 | GRCKLKDFVK | GLSWARSG.. | GNSEQSFS~~ | ~~ |
| *E. nidulans* | GRCtLDDWVE | GLNFARSG.. | GNWktCFTl~ | ~~ |
| *T. thermophilus* | GRCKrDDFVr | GLSFARqG.. | GNWEGCYAas | e~ |
| *T. lanuginosa* | GRCRrDEWIK | GLTFARqG.. | GHWDrCF~~~ | ~~ |
| *M. thermophila* | GmCtLErFIE | SMAFARGN.. | GKWDlCFA~~ | ~~ |
| Consensus Seq. 11 | GRCKLDDFVE | GLSFARSG-- | GNWAECFA-- | -- |

Fig. 8D

```
      M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T  S  G  T       20
      ATGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTCGGTTCCACATCCGGTACC
    1 ---+---------+---------+---------+---------+---------+------  60
      TACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGTGTAGGCCATGG

      A  L  G  P  R  G  N  S  H  S  C  D  T  V  D  G  G  Y  Q  C       40
      GCCTTGGGTCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT
   61 ---+---------+---------+---------+---------+---------+------  120
      CGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCACCAATGGTTACA

      F  P  E  I  S  H  L  W  G  T  Y  S  P  Y  F  S  L  A  D  E       60
      TTCCCAGAAATTTCTCACTTGTGGGGTACCTACTCTCCATACTTCTCTTTGGCAGACGAA
  121 ---+---------+---------+---------+---------+---------+------  180
      AAGGGTCTTTAAAGAGTGAACACCCCATGGATGAGAGGTATGAAGAGAAACCGTCTGCTT

      S  A  I  S  P  D  V  P  D  D  C  R  V  T  F  V  Q  V  L  S       80
      TCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTCGTTCAAGTTTTGTCT
  187 ---+---------+---------+---------+---------+---------+------  240
      AGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAGTTCAAAACAGA

      R  H  G  A  R  Y  P  T  S  S  A  S  K  A  Y  S  A  L  I  E      100
      AGACACGGTGCTAGATACCCAACTTCTTCTGCGTCTAAGGCTTACTCTGCTTTGATTGAA
  241 ---+---------+---------+---------+---------+---------+------  300
      TCTGTGCCACGATCTATGGGTTGAAGAAGACGCAGATTCCGAATGAGACGAAACTAACTT

      A  I  Q  K  N  A  T  A  F  K  G  K  Y  A  F  L  K  T  Y  N      120
      GCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAC
  301 ---+---------+---------+---------+---------+---------+------  360
      CGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTG

      Y  T  L  G  A  D  D  L  T  P  F  G  E  N  Q  M  V  N  S  G      140
      TACACTTTGGGTGCTGACGACTTGACTCCATTCGGTGAAAACCAAATGGTTAACTCTGGT
  361 ---+---------+---------+---------+---------+---------+------  420
      ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACCAATTGAGACCA

      I  K  F  Y  R  R  Y  K  A  L  A  R  K  I  V  P  F  I  R  A      160
      ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTCATTAGAGCT
  421 ---+---------+---------+---------+---------+---------+------  480
      TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTAAGTAATCTCGA

      S  G  S  D  R  V  I  A  S  A  E  K  F  I  E  G  F  Q  S  A      180
      TCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTTTCCAATCTGCT
  481 ---+---------+---------+---------+---------+---------+------  540
      AGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAAAGGTTAGACGA

      K  L  A  D  P  G  S  Q  P  H  Q  A  S  P  V  I  N  V  I  I      200
      AAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTAACGTGATCATT
  541 ---+---------+---------+---------+---------+---------+------  600
      TTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTCGAAGAGGTCAATAATTGCACTAGTAA

      P  E  G  S  G  Y  N  N  T  L  D  H  G  T  C  T  A  F  E  D      220
      CCAGAAGGATCCGGTTACAACAACACTTTGGACCACGGTACTTGTACTGCTTTCGAAGAC
  601 ---+---------+---------+---------+---------+---------+------  660
      GGTCTTCCTAGGCCAATGTTGTTGTGAAACCTGGTGCCATGAACATGACGAAAGCTTCTG
```

Fig. 9A

```
     S  E  L  G  D  D  V  E  A  N  F  T  A  L  F  A  P  A  I  R      240
     TCTGAATTAGGTGACGACGTTGAAGCTAACTTCACTGCTTTGTTCGCTCCAGCTATTAGA
 661 ---+---------+---------+---------+---------+---------+------  720
     AGACTTAATCCACTGCTGCAACTTCGATTGAAGTGACGAAACAAGCGAGGTCGATAATCT

     A  R  L  E  A  D  L  P  G  V  T  L  T  D  E  D  V  V  Y  L      260
     GCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACGAAGACGTTGTTTACTTG
 721 ---+---------+---------+---------+---------+---------+------  780
     CGATCTAACCTTCGACTGAACGGTCCACAATGAAACTGACTGCTTCTGCAACAAATGAAC

     M  D  M  C  P  F  D  T  V  A  R  T  S  D  A  T  E  L  S  P      280
     ATGGACATGTGTCCATTCGACACTGTCGCTAGAACTTCTGACGCTACTGAATTGTCTCCA
 781 ---+---------+---------+---------+---------+---------+------  840
     TACCTGTACACAGGTAAGCTGTGACAGCGATCTTGAAGACTGCGATGACTTAACAGAGGT

     F  C  A  L  F  T  H  D  E  W  I  Q  Y  D  Y  L  Q  S  L  G      300
     TTCTGTGCTTTGTTCACTCACGACGAATGGATCCAATACGACTACTTGCAAAGCTTGGGT
 841 ---+---------+---------+---------+---------+---------+------  900
     AAGACACGAAACAAGTGAGTGCTGCTTACCTAGGTTATGCTGATGAACGTTTCGAACCCA

     K  Y  Y  G  Y  G  A  G  N  P  L  G  P  A  Q  G  V  G  F  A      320
     AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGCT
 901 ---+---------+---------+---------+---------+---------+------  960
     TTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTCGAGTTCCACAACCAAAGCGA

     N  E  L  I  A  R  L  T  H  S  P  V  Q  D  H  T  S  T  N  H      340
     AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC
 961 ---+---------+---------+---------+---------+---------+------ 1020
     TTGCTTAACTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG

     T  L  D  S  N  P  A  T  F  P  L  N  A  T  L  Y  A  D  F  S      360
     ACTTTGGACTCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACGCTGACTTCTCT
1021 ---+---------+---------+---------+---------+---------+------ 1080
     TGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGCGACTGAAGAGA

     H  D  N  T  M  I  S  I  F  F  A  L  G  L  Y  N  G  T  K  P      380
     CACGACAACACTATGATATCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACCAAGCCA
1081 ---+---------+---------+---------+---------+---------+------ 1140
     GTGCTGTTGTGATACTATAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGGTTCGGT

     L  S  T  T  S  V  E  S  I  E  E  T  D  G  Y  S  A  S  W  T      400
     TTGTCTACTACTTCTGTTGAATCTATTGAAGAAACTGACGGTTACTCTGCTTCTTGGACT
1141 ---+---------+---------+---------+---------+---------+------ 1200
     AACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA

     V  P  F  A  A  R  A  Y  V  E  M  M  Q  C  Q  A  E  K  E  P      420
     GTTCCATTCGCTGCTAGAGCTTACGTTGAAATGATGCAATGTCAAGCTGAAAAGGAACCA
1201 ---+---------+---------+---------+---------+---------+------ 1260
     CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACAGTTCGACTTTTCCTTGGT

     L  V  R  V  L  V  N  D  R  V  V  P  L  H  G  C  A  V  D  K      440
     TTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTGCTGTTGACAAG
1261 ---+---------+---------+---------+---------+---------+------ 1320
     AACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACGACAACTGTTC

     L  G  R  C  K  R  D  D  F  V  E  G  L  S  F  A  R  S  G  G      460
```

Fig. 9B

```
     TTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTAGATCTGGTGGT
1321 ---+---------+---------+---------+---------+---------+------ 1380
     AACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA

     N  W  A  E  C  F  A  *   467
     AACTGGGCTGAATGTTTCGCTTAA
1381 ---+---------+---------+ 1410
     TTGACCCGACTTACAAAGCGAATT
```

Fig. 9C

```
     M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T  S  G  T       20
     ATGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTCGGTTCCACATCCGGTACC     60
1    ---------+---------+---------+---------+---------+---------+
     TACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGTGTAGGCCATGG

     A  L  G  P  R  G  N  S  H  S  C  D  T  V  D  G  G  Y  Q  C       40
     GCCTTGGGTCCTCGTGGTAACTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT     120
61   ---------+---------+---------+---------+---------+---------+
     CGGAACCCAGGAGCACCATTGAGAGTGAGAACACTGTGACAACTGCCACCAATGGTTACA

A    F  P  E  I  S  H  L  W  G  T  Y  S  P  F  F  S  L  A  D  E       60
     TTCCCAGAAATTTCTCACTTGTGGGGTACATACTCTCCATTCTTCTCTTTGGCTGACGAA     180
121  ---------+---------+---------+---------+---------+---------+
     AAGGGTCTTTAAAGAGTGAACACCCCATGTATGAGAGGTAAGAAGAGAAACCGACTGCTT

     S  A  I  S  P  D  V  P  K  G  C  R  V  T  F  V  Q  V  L  S       80
     TCTGCTATTTCTCCAGACGTTCCAAAGGGTTGTAGAGTTACTTTCGTTCAAGTTTTGTCT     240
181  ---------+---------+---------+---------+---------+---------+
     AGACGATAAAGAGGTCTGCAAGGTTTCCCAACATCTCAATGAAAGCAAGTTCAAAACAGA

     R  H  G  A  R  Y  P  T  S  S  A  S  K  A  Y  S  A  L  I  E       100
     AGACACGGTGCTAGATACCCAACTTCTTCTGCGTCTAAGGCGTACTCTGCTTTGATTGAA     300
241  ---------+---------+---------+---------+---------+---------+
     TCTGTGCCACGATCTATGGGTTGAAGAAGACGCAGATTCCGCATGAGACGAAACTAACTT

     A  I  Q  K  N  A  T  A  F  K  G  K  Y  A  F  L  K  T  Y  N       120
     GCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAC     360
301  ---------+---------+---------+---------+---------+---------+
     CGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTG

A    Y  T  L  G  A  D  D  L  T  P  F  G  E  Q  Q  M  V  N  S  G       140
     TACACTTTGGGTGCTGACGACTTGACTCCATTCGGTGAACAACAAATGGTTAACTCTGGT     420
361  ---------+---------+---------+---------+---------+---------+
     ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTGTTGTTTACCAATTGAGACCA

     I  K  F  Y  R  R  Y  K  A  L  A  R  K  I  V  P  F  I  R  A       160
     ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTCATTAGAGCT     480
421  ---------+---------+---------+---------+---------+---------+
     TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTAAGTAATCTCGA

     S  G  S  D  R  V  I  A  S  A  E  K  F  I  E  G  F  Q  S  A       180
     TCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTTTCCAATCTGCT     540
481  ---------+---------+---------+---------+---------+---------+
     AGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAAAGGTTAGACGA

     K  L  A  D  P  G  A  N  P  H  Q  A  S  P  V  I  N  V  I  I       200
     AAGTTGGCTGACCCAGGTGCTAACCCACACCAAGCTTCTCCAGTTATTAACGTTATTATT     600
541  ---------+---------+---------+---------+---------+---------+
     TTCAACCGACTGGGTCCACGATTGGGTGTGGTTCGAAGAGGTCAATAATTGCAATAATAA

     P  E  G  A  G  Y  N  N  T  L  D  H  G  L  C  T  A  F  E  E       220
     CCAGAAGGTGCTGGTTACAACAACACTTTGGACCACGGTTTGTGTACTGCTTTCGAAGAA     660
601  ---------+---------+---------+---------+---------+---------+
     GGTCTTCCACGACCAATGTTGTTGTGAAACCTGGTGCCAAACACATGACGAAAGCTTCTT
```

Fig. 10A

```
     S  E  L  G  D  D  V  E  A  N  F  T  A  V  F  A  P  P  I  R      240
     TCTGAATTGGGTGACGACGTTGAAGCTAACTTCACTGCTGTTTTCGCTCCACCAATTAGA   720
 661 ---------+---------+---------+---------+---------+---------+
     AGACTTAACCCACTGCTGCAACTTCGATTGAAGTGACGACAAAAGCGAGGTGGTTAATCT

     A  R  L  E  A  H  L  P  G  V  N  L  T  D  E  D  V  V  N  L      260
     GCTAGATTGGAAGCTCACTTGCCAGGTGTTAACTTGACTGACGAAGACGTTGTTAACTTG   780
 721 ---------+---------+---------+---------+---------+---------+
     CGATCTAACCTTCGAGTGAACGGTCCACAATTGAACTGACTGCTTCTGCAACAATTGAAC

     M  D  M  C  P  F  D  T  V  A  R  T  S  D  A  T  Q  L  S  P      280
     ATGGACATGTGTCCATTCGACACTGTTGCTAGAACTTCTGACGCTACTCAATTGTCTCCA   840
 781 ---------+---------+---------+---------+---------+---------+
     TACCTGTACACAGGTAAGCTGTGACAACGATCTTGAAGACTGCGATGAGTTAACAGAGGT

     F  C  D  L  F  T  H  D  E  W  I  Q  Y  D  Y  L  Q  S  L  G      300
     TTCTGTGACTTGTTCACTCACGACGAATGGATTCAATACGACTACTTGCAATCTTTGGGT   900
 841 ---------+---------+---------+---------+---------+---------+
     AAGACACTGAACAAGTGAGTGCTGCTTACCTAAGTTATGCTGATGAACGTTAGAAACCCA

     K  Y  Y  G  Y  G  A  G  N  P  L  G  P  A  Q  G  V  G  F  V      320
     AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGTT   960
 901 ---------+---------+---------+---------+---------+---------+
     TTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTCGAGTTCCACAACCAAAGCAA

     N  E  L  I  A  R  L  T  H  S  P  V  Q  D  H  T  S  T  N  H      340
     AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC   1020
 961 ---------+---------+---------+---------+---------+---------+
     TTGCTTAACTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG

     T  L  D  S  N  P  A  T  F  P  L  N  A  T  L  Y  A  D  F  S      360
     ACTTTGGACTCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACGCTGACTTCTCT   1080
1021 ---------+---------+---------+---------+---------+---------+
     TGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGCGACTGAAGAGA

     H  D  N  T  M  V  S  I  F  F  A  L  G  L  Y  N  G  T  K  P      380
     CACGACAACACTATGGTTTCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACTAAGCCA   1140
1081 ---------+---------+---------+---------+---------+---------+
     GTGCTGTTGTGATACCAAAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGATTCGGT

     L  S  T  T  S  V  E  S  I  E  E  T  D  G  Y  S  A  S  W  T      400
     TTGTCTACTACTTCTGTTGAATCTATTGAAGAAACTGACGGTTACTCTGCTTCTTGGACT   1200
1141 ---------+---------+---------+---------+---------+---------+
     AACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA

     V  P  F  A  A  R  A  Y  V  E  M  M  Q  C  E  A  E  K  E  P      420
     GTTCCATTCGCTGCTAGAGCTTACGTTGAAATGATGCAATGTGAAGCTGAAAAGGAACCA   1260
1201 ---------+---------+---------+---------+---------+---------+
     CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACACTTCGACTTTTCCTTGGT

     L  V  R  V  L  V  N  D  R  V  V  P  L  H  G  C  G  V  D  K      440
     TTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTGGTGTTGACAAG   1320
1261 ---------+---------+---------+---------+---------+---------+
     AACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACCACAACTGTTC

     L  G  R  C  K  R  D  D  F  V  E  G  L  S  F  A  R  S  G  G      460
```

Fig. 10B

```
     TTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTAGATCTGGTGGT
1321 ---------+---------+---------+---------+---------+---------+ 1380
     AACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA

     N  W  E  E  C  F  A  *    467
     AACTGGGAAGAATGTTTCGCTTAA
1381 ---------+---------+---- 1404
     TTGACCCTTCTTACAAAGCGAATT
```

Fig. 10C

```
    M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T  S  G  T    20
    ATGGGGGTTTTCGTCGTTCTATTATCTATCGCGACTCTGTTCGGCAGCACATCGGGCACT
1   ---------+---------+---------+---------+---------+---------+ 60
    TACCCCCAAAAGCAGCAAGATAATAGATAGCGCTGAGACAAGCCGTCGTGTAGCCCGTGA

    A  L  G  P  R  G  N  H  S  K  S  C  D  T  V  D  L  G  Y  Q    40
    GCGCTGGGCCCCCGTGGAAATCACTCCAAGTCCTGCGATACGGTAGACCTAGGGTACCAG
61  ---------+---------+---------+---------+---------+---------+ 120
    CGCGACCCGGGGGCACCTTTAGTGAGGTTCAGGACGCTATGCCATCTGGATCCCATGGTC

    C  S  P  A  T  S  H  L  W  G  T  Y  S  P  Y  F  S  L  E  D    60
    TGCTCCCCTGCGACTTCTCATCTATGGGGCACGTACTCGCCATaCTTTTCGCTCGAGGAC
121 ---------+---------+---------+---------+---------+---------+ 180
    ACGAGGGGACGCTGAAGAGTAGATACCCCGtgCATGAGCGGTAtGAAAAGCGAGCTCCTG

    E  L  S  V  S  S  K  L  P  K  D  C  R  I  T  L  V  Q  V  L    80
    GAGCTGTCCGTGTCGAGTAAGCTTCCCAAGGATTGCCGGATCACCTTGGTACAGGTGCTA
181 ---------+---------+---------+---------+---------+---------+ 240
    CTCGACAGGCACAGCTCATTCGAAGGGTTCCTAACGGCCTAGTGGAACCATGTCCACGAT

    S  R  H  G  A  R  Y  P  T  S  S  K  S  K  K  Y  K  K  L  I    100
    TCGCGCCATGGAGCGCGGTACCCAACCAGCTCCAAGAGCAAAAAGTATAAGAAGCTTaTt
241 ---------+---------+---------+---------+---------+---------+ 300
    AGCGCGGTACCTCGCGCCATGGGTTGGTCGAGGTTCTCGTTTTTCATATTCTTCGAAtAa

    T  A  I  Q  A  N  A  T  D  F  K  G  K  Y  A  F  L  K  T  Y    120
    ACGGCGATCCAGGCCAATGCCACCGACTTCAAGGGCAAGTacGCCTTTTTGAAGACGTAC
301 ---------+---------+---------+---------+---------+---------+ 360
    TGCCGCTAGGTCCGGTTACGGTGGCTGAAGTTCCCGTTCAtgCGGAAAAACTTCTGCATG

    N  Y  T  L  G  A  D  D  L  T  P  F  G  E  Q  Q  L  V  N  S    140
    AACTATACTCTGGGTGCGGATGACCTCACTCCCTTTGGGGAGCAGCAGCTGGTGAACTCG
361 ---------+---------+---------+---------+---------+---------+ 420
    TTGATATGAGACCCACGCCTACTGGAGTGAGGGAAACCCCTCGTCGTCGACCACTTGAGC

    G  I  K  F  Y  Q  R  Y  K  A  L  A  R  S  V  V  P  F  I  R    160
    GGCATCAAGTTCTACCAGAGGTACAAGGCTCTGGCGCGCAGTGTGGTGCCGTTTATTCGC
421 ---------+---------+---------+---------+---------+---------+ 480
    CCGTAGTTCAAGATGGTCTCCATGTTCCGAGACCGCGCGTCACACCACGGCAAATAAGCG

    A  S  G  S  D  R  V  I  A  S  G  E  K  F  I  E  G  F  Q  Q    180
    GCCTCAGGCTCGGACCGGGTTATTGCTTCGGGAGAGAAGTTCATCGAGGGGTTCCAGCAG
481 ---------+---------+---------+---------+---------+---------+ 540
    CGGAGTCCGAGCCTGGCCCAATAACGAAGCCCTCTCTTCAAGTAGCTCCCCAAGGTCGTC

    A  K  L  A  D  P  G  A  T  N  R  A  A  P  A  I  S  V  I  I    200
    GCGAAGCTGGCTGATCCTGGCGCGACGAACCGCGCCGCTCCGGCGATTAGTGTGATTATT
541 ---------+---------+---------+---------+---------+---------+ 600
    CGCTTCGACCGACTAGGACCGCGCTGCTTGGCGCGGCGAGGCCGCTAATCACACTAATAA

    P  E  S  E  T  F  N  N  T  L  D  H  G  V  C  T  K  F  E  A    220
    CCGGAGAGCGAGACGTTCAACAATACGCTGGACCACGGTGTGTGCACGAAGTTTGAGGCG
601 ---------+---------+---------+---------+---------+---------+ 660
    GGCCTCTCGCTCTGCAAGTTGTTATGCGACCTGGTGCCACACACGTGCTTCAAACTCCGC
```

Fig. 11A

```
     S  Q  L  G  D  E  V  A  A  N  F  T  A  L  F  A  P  D  I  R    240
     AGTCAGCTGGGAGATGAGGTTGCGGCCAATTTCACTGCGCTCTTTGCACCCGACATCCGA
 661 ---------+---------+---------+---------+---------+---------+ 720
     TCAGTCGACCCTCTACTCCAACGCCGGTTAAAGTGACGCGAGAAACGTGGGCTGTAGGCT

     A  R  L  E  K  H  L  P  G  V  T  L  T  D  E  D  V  V  S  L    260
     GCTCGCCtCGAGAAGCATCTTCCTGGCGTGACGCTGACAGACGAGGACGTTGTCAGTCTA
 721 ---------+---------+---------+---------+---------+---------+ 780
     CGAGCGGaGCTCTTCGTAGAAGGACCGCACTGCGACTGTCTGCTCCTGCAACAGTCAGAT

     M  D  M  C  P  F  D  T  V  A  R  T  S  D  A  S  Q  L  S  P    280
     ATGGACATGTGTcCGTTTGATACGGTAGCGCGCACCAGCGACGCAAGTCAGCTGTCACCG
 781 ---------+---------+---------+---------+---------+---------+ 840
     TACCTGTACACAgGCAAACTATGCCATCGCGCGTGGTCGCTGCGTTCAGTCGACAGTGGC

     F  C  Q  L  F  T  H  N  E  W  K  K  Y  D  Y  L  Q  S  L  G    300
     TTCTGTCAACTCTTCACTCACAATGAGTGGAAGAAGTACgACTACCTTCAGTCCTTGGGC
 841 ---------+---------+---------+---------+---------+---------+ 900
     AAGACAGTTGAGAAGTGAGTGTTACTCACCTTCTTCATGcTGATGGAAGTCAGGAACCCG

     K  Y  Y  G  Y  G  A  G  N  P  L  G  P  A  Q  G  I  G  F  T    320
     AAGTACTACGGCTACGGCGCAGGCAACCCTCTGGGACCGGCTCAGGGGATAGGGTTCACC
 901 ---------+---------+---------+---------+---------+---------+ 960
     TTCATGATGCCGATGCCGCGTCCGTTGGGAGACCCTGGCCGAGTCCCCTATCCCAAGTGG

     N  E  L  I  A  R  L  T  R  S  P  V  Q  D  H  T  S  T  N  S    340
     AACGAGCTGATTGCCCGGTTGACgCGTTCGCCAGTGCAGGACCACACCAGCACTAACTCG
 961 ---------+---------+---------+---------+---------+---------+ 1020
     TTGCTCGACTAACGGGCCAACTGcGCAAGCGGTCACGTCCTGGTGTGGTCGTGATTGAGC

     T  L  V  S  N  P  A  T  F  P  L  N  A  T  M  Y  V  D  F  S    360
     ACTCTAGTCTCCAACCCGGCCACCTTCCCGTTGAACGCTACCATGTACGTCGACTTTTCA
1021 ---------+---------+---------+---------+---------+---------+ 1080
     TGAGATCAGAGGTTGGGCCGGTGGAAGGGCAACTTGCGATGGTACATGCAGCTGAAAAGT

     H  D  N  S  M  V  S  I  F  F  A  L  G  L  Y  N  G  T  E  P    380
     CACGACAACAGCATGGTTTCCATCTTCTTTGCATTGGGCCTGTACAACGGCACTGAACCC
1081 ---------+---------+---------+---------+---------+---------+ 1140
     GTGCTGTTGTCGTACCAAAGGTAGAAGAAACGTAACCCGGACATGTTGCCGTGACTTGGG

     L  S  R  T  S  V  E  S  A  K  E  L  D  G  Y  S  A  S  W  V    400
     TTGTCCCGGACCTCGGTGGAAAGCGCCAAGGAATTGGATGGGTATTCTGCATCCTGGGTG
1141 ---------+---------+---------+---------+---------+---------+ 1200
     AACAGGGCCTGGAGCCACCTTTCGCGGTTCCTTAACCTACCCATAAGACGTAGGACCCAC

     V  P  F  G  A  R  A  Y  F  E  T  M  Q  C  K  S  E  K  E  P    420
     GTGCCTTTCGGCGCGCGAGCCTACTTCGAGACGATGCAATGCAAGTCGGAAAAGGAGCCT
1201 ---------+---------+---------+---------+---------+---------+ 1260
     CACGGAAAGCCGCGCGCTCGGATGAAGCTCTGCTACGTTACGTTCAGCCTTTTCCTCGGA

     L  V  R  A  L  I  N  D  R  V  V  P  L  H  G  C  D  V  D  K    440
     CTTGTTCGCGCTTTGATTAATGACCGGGTTGTGCCACTGCATGGCTGCGATGTGGACAAG
1261 ---------+---------+---------+---------+---------+---------+ 1320
     GAACAAGCGCGAAACTAATTACTGGCCCAACACGGTGACGTACCGACGCTACACCTGTTC

     L  G  R  C  K  L  N  D  F  V  K  G  L  S  W  A  R  S  G  G    460
```

Fig. 11B

```
     CTGGGGCGATGCAAGCTGAATGACTTTGTCAAGGGATTGAGTTGGGCCAGATCTGGGGGC
1321 ---------+---------+---------+---------+---------+---------+ 1380
     GACCCCGCTACGTTCGACTTACTGAAACAGTTCCCTAACTCAACCCGGTCTAGACCCCCG

     N  W  G  E  C  F  S  *   467
     AACTGGGGAGAGTGCTTTAGTTGA
1381 ---------+---------+---- 1404
     TTGACCCCTCTCACGAAATCAACT
```

Fig. 11C

```
CP-1
    Eco RI M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T
   TATATGAATTCATGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTCGGTTCCA
 1 ---------+---------+---------+---------+---------+---------+ 60
   ATATACTTAAGTACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGT

     S  G  T  A  L  G  P  R  G  N  S  H  S  C  D  T  V  D  G  G
   CATCCGGTACCGCCTTGGGTCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTG
61 ---------+---------+---------+---------+---------+---------+ 120
   GTAGGCCATGGCGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCAC
        CP-2
            CP-3
     Y  Q  C  F  P  E  I  S  H  L  W  G  Q  Y  S  P  Y  F  S  L
    GTTACCAATGTTTCCCAGAAATTTCTCACTTGTGGGGTCAATACTCTCCATACTTCTCTT
121 ---------+---------+---------+---------+---------+---------+ 180
    CAATGGTTACAAAGGGTCTTTAAAGAGTGAACACCCCAGTTATGAGAGGTATGAAGAGAA

     E  D  E  S  A  I  S  P  D  V  P  D  D  C  R  V  T  F  V  Q
    TGGAAGACGAATCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTCGTTC
181 ---------+---------+---------+---------+---------+---------+ 240
    ACCTTCTGCTTAGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAG
              CP-4.7
                  CP-5.7
     V  L  S  R  H  G  A  R  Y  P  T  D  S  K  G  K  K  Y  S  A
    AAGTTTTGTCTAGACACGGTGCTAGATACCCAACTgacTCTAAGggtAAGaagTACTCTG
241 ---------+---------+---------+---------+---------+---------+ 300
    TTCAAAACAGATCTGTGCCACGATCTATGGGTTGActgAGATTCccaTTCttcATGAGAC

     L  I  E  A  I  Q  K  N  A  T  A  F  K  G  K  Y  A  F  L  K
    CTTTGATTGAAGCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGA
301 ---------+---------+---------+---------+---------+---------+ 360
    GAAACTAACTTCGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACT
                                      CP-6
                                          CP-7
     T  Y  N  Y  T  L  G  A  D  D  L  T  P  F  G  E  N  Q  M  V
    AGACTTACAACTACACTTTGGGTGCTGACGACTTGACTCCATTCGGTGAAAACCAAATGG
361 ---------+---------+---------+---------+---------+---------+ 420
    TCTGAATGTTGATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACC

     N  S  G  I  K  F  Y  R  R  Y  K  A  L  A  R  K  I  V  P  F
    TTAACTCTGGTATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCAT
421 ---------+---------+---------+---------+---------+---------+ 480
    AATTGAGACCATAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTA
                                               CP-8.7
                                                   CP-9
     I  R  A  S  G  S  S  R  V  I  A  S  A  E  K  F  I  E  G  F
    TCATTAGAGCTTCTGGTTCTtctAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTT
481 ---------+---------+---------+---------+---------+---------+ 540
    AGTAATCTCGAAGACCAAGAagaTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAA

     Q  S  A  K  L  A  D  P  G  S  Q  P  H  Q  A  S  P  V  I  D
    TCCAATCTGCTAAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTG
541 ---------+---------+---------+---------+---------+---------+ 600
    AGGTTAGACGATTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTCGAAGAGGTCAATAAC
```

Fig. 12A

```
                                                   CP-10.7
                                                       CP-11.7
     V  I  I  S  E  A  S  S  Y  N  N  T  L  D  P  G  T  C  T  A
    ACGTTATTATTtctGAcgctTCTtctTACAACAACACTTTGGACccaGGTACTTGTACTG
601 ---------+---------+---------+---------+---------+---------+ 660
    TGCAATAATAAagaCTgcgaAGGagaATGTTGTTGTGAAACCTGggtCCATGAACATGAC
```

Fig. 12B

```
      F  E  D  S  E  L  A  D  T  V  E  A  N  F  T  A  L  F  A  P
     CTTTCGAAGACTCTGAATTGgctGACactGTTGAAGCTAACTTCACTGCTTTGTTCGCTC
 661 ---------+---------+---------+---------+---------+---------+ 720
     GAAAGCTTCTGAGACTTAACcgaCTGtgaCAACTTCGATTGAAGTGACGAAACAAGCGAG
                                                         CP-12.7

      A  I  R  A  R  L  E  A  D  L  P  G  V  T  L  T  D  T  E  V
     CAGCTATTAGAGCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACactgaaG
 721 ---------+---------+---------+---------+---------+---------+ 780
     GTCGATAATCTCGATCTAACCTTCGACTGAACGGTCCACAATGAAACTGACTGtgacttC

     CP-13.7
      T  Y  L  M  D  M  C  S  F  E  T  V  A  R  T  S  D  A  T  E
     TTactTACTTGATGGACATGTGTtctTTCGAAACTGTTGCTAGAACTTCTGACGCTACTG
 781 ---------+---------+---------+---------+---------+---------+ 840
     AAtgaATGAACTACCTGTACACAagaAAGCTTTGACAACGATCTTGAAGACTGCGATGAC

      L  S  P  F  C  A  L  F  T  H  D  E  W  R  H  Y  D  Y  L  Q
     AATTGTCTCCATTCTGTGCTTTGTTCACTCACGACGAATGGAGAcacTACGACTACTTGC
 841 ---------+---------+---------+---------+---------+---------+ 900
     TTAACAGAGGTAAGACACGAAACAAGTGAGTGCTGCTTACCTCTgtgATGCTGATGAACG
          CP-14.7
             CP-15.7
      S  L  K  K  Y  Y  G  H  G  A  G  N  P  L  G  P  T  Q  G  V
     AATCTTTGaagAAGTACTACGGTcacGGTGCTGGTAACCCATTGGGTCCAactCAAGGTG
 901 ---------+---------+---------+---------+---------+---------+ 960
     TTAGAAACttcTTCATGATGCCAgtgCCACGACCATTGGGTAACCCAGGTtgaGTTCCAC

      G  F  A  N  E  L  I  A  R  L  T  R  S  P  V  Q  D  H  T  S
     TTGGTTTCGCTAACGAATTGATTGCTAGATTGACTAGATCTCCAGTTCAAGACCACACTT
 961 ---------+---------+---------+---------+---------+---------+ 1020
     AACCAAAGCGATTGCTTAACTAACGATCTAACTGATCTAGAGGTCAAGTTCTGGTGTGAA
                         CP-16
                           CP-17.7
      T  N  H  T  L  D  S  N  P  A  T  F  P  L  N  A  T  L  Y  A
     CTACTAACCACACTTTGGACTCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACG
1021 ---------+---------+---------+---------+---------+---------+ 1080
     GATGATTGGTGTGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGC

      D  F  S  H  D  N  G  I  I  S  I  F  F  A  L  G  L  Y  N  G
     CTGACTTCTCTCACGACAACggtattATTTCTATTTTCTTCGCTTTGGGTTTGTACAACG
1081 ---------+---------+---------+---------+---------+---------+ 1140
     GACTGAAGAGAGTGCTGTTGccataaTAAAGATAAAAGAAGCGAAACCCAAACATGTTGC
                               CP-18.7
                                  CP-19.7
      T  A  P  L  S  T  T  S  V  E  S  I  E  E  T  D  G  Y  S  S
     GTACTGCTCCATTGTCTACTACTTCTGTTGAATCTATTGAAGAAACTGACGGTTACTCTt
1141 ---------+---------+---------+---------+---------+---------+ 1200
     CATGACGAGGTAACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGAGAa

      A  W  T  V  P  F  A  S  R  A  Y  V  E  M  M  Q  C  Q  A  E
     ctgctTGGACTGTTCCATTCgcttctAGAGCTTACGTTGAAATGATGCAATGTCAAGCTG
1201 ---------+---------+---------+---------+---------+---------+ 1260
     gacgaACCTGACAAGGTAAGcgaagaTCTCGAATGCAACTTTACTACGTTACAGTTCGAC
                                             CP-20
                                                CP-21
```

Fig. 12C

```
      K  E  P  L  V  R  V  L  V  N  D  R  V  V  P  L  H  G  C  A
     AAAAGGAACCATTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTG
1261 ---------+---------+---------+---------+---------+---------+ 1320
     TTTTCCTTGGTAACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACAC
```

Fig. 12D

```
     V  D  K  L  G  R  C  K  R  D  D  F  V  E  G  L  S  F  A  R
     CTGTTGACAAGTTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTA
1321 ---------+---------+---------+---------+---------+---------+ 1380
     GACAACTGTTCAACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGAT
                                                        CP-22

     S  G  G  N  W  A  E  C  F  A  *  Eco RI
     GATCTGGTGGTAACTGGGCTGAATGTTTCGCTTAAGAATTCATATA
1381 ---------+---------+---------+---------+------ 1426
     CTAGACCACCATTGACCCGACTTACAAAGCGAATTCTTAAGTATAT
```

Fig. 12E

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 99/00154

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC6: A23K 1/165, A01H 5/00, C12N 9/16
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| X | EP 0619369 A1 (AVEVE N.V.), 12 October 1994 (12.10.94), See page 5, lines 11-15; page 7, lines 1-3; and claims 20-21<br>-- | 1-14 |
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 6 July 1999 | 13 -07- 1999 |
| Name and mailing address of the ISA/<br>Swedish Patent Office<br>Box 5055, S-102 42 STOCKHOLM<br>Facsimile No. +46 8 666 02 86 | Authorized officer<br>Carolina Palmcrantz/Els<br>Telephone No. +46 8 782 25 00 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 99/00154

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01/06/99

International application No.
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